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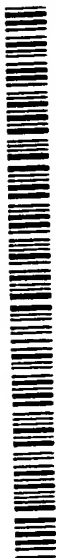
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(54) Title: MELANIN CONCENTRATING HORMONE RECEPTOR CHIMERIC AND FUSION PROTEINS

(57) Abstract: The present invention features melanin concentrating hormone receptor (MCH-R) chimeric and fusion proteins. MCH-R chimeric proteins comprise an MCH-R polypeptide region made up of at least two or more polypeptide regions characteristic of MCH-R found in different species. MCH-R fusion proteins comprise an MCH-R polypeptide region and a fluorescent protein region.

TITLE OF THE INVENTION  
MELANIN CONCENTRATING HORMONE RECEPTOR CHIMERIC AND  
FUSION PROTEINS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority to provisional application U.S. Serial No. 60/189,698, filed March 15, 2000, hereby incorporated by reference herein.

BACKGROUND OF THE INVENTION

10 The references cited herein are not admitted to be prior art to the claimed invention.

Neuropeptides present in the hypothalamus play a major role in mediating the control of body weight. (Flier *et al.*, 1998. *Cell*, 92, 437-440.) Melanin-concentrating hormone (MCH) is a cyclic 19-amino acid neuropeptide synthesized as  
15 part of a larger pre-prohormone precursor in the hypothalamus which also encodes neuropeptides NEI and NGE. (Nahon *et al.*, 1990. *Mol. Endocrinol.* 4, 632-637.) MCH was first identified in salmon pituitary, and in fish MCH affects melanin aggregation thus affecting skin pigmentation. In trout and in eels MCH has also been shown to be involved in stress induced or CRF-stimulated ACTH release. (Kawauchi  
20 *et al.*, 1983. *Nature* 305, 321-323.)

In humans two genes encoding MCH have been identified that are expressed in the brain. (Breton *et al.*, 1993. *Mol. Brain Res.* 18, 297-310.) In mammals MCH has been localized primarily to neuronal cell bodies of the hypothalamus which are implicated in the control of food intake, including perikarya  
25 of the lateral hypothalamus and zona inertia. (Knigge *et al.*, 1996. *Peptides* 17, 1063-1073.)

Pharmacological and genetic evidence suggest that the primary mode of MCH action is to promote feeding (orexigenic). MCH mRNA is up regulated in fasted mice and rats and in the *ob/ob* mouse. (Qu *et al.*, 1996. *Nature* 380, 243-247.)  
30 Injection of MCH centrally (ICV) stimulates food intake and MCH antagonizes the hypophagic effects seen with  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ MSH). (Qu *et al.*, 1996. *Nature* 380, 243-247.) MCH-deficient mice are lean, hypophagic, and have increased metabolic rate. (Shimada *et al.*, 1998. *Nature* 396, 670-673.)

MCH action is not limited to modulation of food intake as effects on  
35 the hypothalamic-pituitary-axis have been reported. (Nahon 1994. *Critical Rev. in*

*Neurobiol.* 8, 221-262.) MCH may be involved in the body response to stress as MCH can modulate the stress-induced release of CRF from the hypothalamus and ACTH from the pituitary. In addition, MCH neuronal systems may be involved in reproductive or maternal function.

5               Several references describe a receptor that is indicated to bind MCH. (Chambers *et al.*, 1999. *Nature* 400, 261-265; Saito *et al.*, 1999. *Nature* 400, 265-269; Bächner *et al.*, 1999. *FEBS Letters* 457:522-524; Shimomura *et al.*, 1999. *Biochemical and Biophysical Research Communications* 261, 622-626; and Lembo *et al.*, 1999. *Nat. Cell Biol.* 1, 267-271.)

10

#### SUMMARY OF THE INVENTION

The present invention features melanin concentrating hormone receptor (MCH-R) chimeric and fusion proteins. MCH-R chimeric proteins comprise an MCH-R polypeptide region made up of at least two or more polypeptide regions  
15               characteristic of MCH-R found in different species. MCH-R fusion proteins comprise an MCH-R polypeptide region and a fluorescent protein region.

An MCH-R polypeptide region provides a functional G-protein coupled receptor region able to bind MCH and transduce an intracellular signal. Examples of MCH-R polypeptide regions include naturally occurring MCH-R,  
20               chimeric MCH-R containing two or more regions from naturally occurring MCH-R, and functional derivatives thereof.

Reference to the terms "characteristic" and "derivatives thereof" describe a relationship to a reference sequence. In both cases, there is at least about 75% sequence similarity to the reference sequence.

25               Thus, a first aspect of the present invention describes a fusion protein comprising (a) an MCH-R polypeptide region and (b) a fluorescent polypeptide region. The fluorescent polypeptide region is joined directly, or through a polypeptide linker, to the carboxy side of the MCH-R polypeptide region.

Another aspect of the present invention describes an MCH-R chimeric  
30               protein. The protein comprises: (a) an MCH-R binding region characteristic of a human MCH-R, (b) a transmembrane domain characteristic of a non-human MCH-R, and (c) an intracellular domain characteristic of a non-human MCH-R.

Another aspect of the present invention describes a nucleic acid encoding for an MCH-R fusion protein or an MCH-R chimeric protein described  
35               herein. Such nucleic acid comprises either a contiguous nucleotide sequence that



codes for the protein or a sequence that is processed by a host cell to produce a contiguous nucleotide sequence encoding for the protein. Processing of a nucleic acid sequence to produce a contiguous nucleotide sequence encoding for a protein can occur by the splicing together of exons resulting in intron removal.

5 Another aspect of the present invention describes an expression vector comprising a nucleic acid encoding for an MCH-R fusion protein or an MCH-R chimeric protein described herein.

Another aspect of the present invention describes a recombinant cell comprising nucleic acid encoding for an MCH-R fusion protein or an MCH-R  
10 chimeric protein described herein. The nucleic acid may be part of the host genome or may exist independently of the host genome.

Another aspect of the present invention describes a non-human transgenic animal comprising nucleic acid encoding for an MCH-R fusion protein or an MCH-R chimeric protein described herein.

15 Another aspect of the present invention describes a method for assaying for MCH-R active compounds by measuring the effect of a test preparation on one or more MCH-R activities. The method is performed using either an MCH-R fusion protein or an MCH-R chimeric protein described herein.

Other features and advantages of the present invention are apparent  
20 from the additional descriptions provided herein including the different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention.

25

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates aequorin assay results comparing a mouse MCH-R fusion with a human wild type MCH-R and a CMV-EGFP control.

Figure 2 illustrates a cAMP flashplate assay of CHO cell clones stably  
30 expressing mMCH-1R-EGFP. Cells from individual clones were dissociated in enzyme free media and stimulated for 15 minutes at 37°C with human MCH at the indicated concentrations in the presence of 10 µM forskolin. Cells were then lysed and assayed for bound [<sup>125</sup>I]cAMP. Mouse MCH-1R-EGFP clones exhibited EC<sub>50</sub> values (0.1111, 0.1255, 0.1291, or 0.2304 nM) indistinguishable from that of a CHO  
35 cell clone expressing the wild-type human short isoform of MCH-1R (0.1282 nM).

Figure 3 illustrates a cAMP flashplate assay of CHO cell clones stably expressing human short/mouse species chimeric MCH-1R-EGFP. Cells from individual clones were dissociated in enzyme free media and stimulated for 15 minutes at 37°C with human MCH at the indicated concentrations in the presence of 10 µM forskolin. Cells were then lysed and assayed for bound [<sup>125</sup>I]cAMP. Human short/mouse species chimeric MCH-1R-EGFP clones exhibited EC50 values (0.0366, 0.0462, 0.2117, or 0.2499 nM) indistinguishable from that of a CHO cell clone expressing the wild-type human short isoform of MCH-1R (0.1137 nM).

## 10 DETAILED DESCRIPTION OF THE INVENTION

The present invention features MCH-R chimeric and fusion proteins. Such proteins have a variety of different uses including being used as a research tool to study MCH-R function and dynamics, and being used to screen for MCH-R agonists and antagonists.

15 The MCH-R provides a target to achieve different beneficial effects in a patient. Preferably, MCH-R activity is modulated to achieve one or more of the following: weight loss, weight gain, treat cancer (*e.g.*, colon or breast), reduce pain, treat diabetes, reduce stress, or treat sexual dysfunction.

Modulation of MCH-R activity can be achieved by evoking a response at the MCH receptor or by altering a response evoked by an MCH receptor agonist or antagonist. Compounds modulating MCH-R receptor activity include agonists, antagonists, and allosteric modulators. Generally, MCH-R antagonists and allosteric modulators negatively affecting activity will be used to achieve weight loss, treat cancer (*e.g.*, colon or breast), reduce pain, reduce stress, or treat sexual dysfunction; and MCH-R agonists and allosteric modulators positively affecting activity will be used to produce a weight gain.

Preferably, MCH-R activity is modulated to achieve a weight loss or to treat diabetes in a patient. Diabetes mellitus can be treated by modulating MCH-R activity to achieve, for example, one or both of the following: enhancing glucose tolerance or decreasing insulin resistance.

Excessive body weight is a contributing factor to different diseases, including hypertension, diabetes, dyslipidemias, cardiovascular disease, gall stones, osteoarthritis, and certain forms of cancers. Bringing about a weight loss can be used, for example, to reduce the likelihood of such diseases and as part of a treatment for such diseases. Weight reduction can be achieved by modulating MCH-R activity to

obtain, for example, one or more of the following effects: reducing appetite, increasing metabolic rate, reducing fat intake, or reducing carbohydrate craving.

Increasing body weight is particularly useful for a patient having a disease or disorder, or under going a treatment, accompanied by weight loss.

- 5 Examples of diseases or disorders accompanied by weight loss include anorexia, AIDS, wasting, cachexia, and frail elderly. Examples of treatments accompanied by weight loss include chemotherapy and radiation therapy.

#### MCH-R Chimeric Proteins

- 10 MCH-R chimeric proteins contain an MCH-R polypeptide region made up by at least two or more polypeptide regions characteristic of MCH-R found in different species. The different polypeptide regions that are present provide for an N-terminal extracellular domain; a transmembrane domain made up of transmembrane regions, extracellular loop regions, and intracellular loop regions; and an intracellular  
15 carboxy terminus domain. Examples of MCH-R amino acid sequences include the following: SEQ. ID. NO. 1 (human MCH1R long form), SEQ. ID. NO. 2 (human MCH1R short form), and SEQ. ID. NO. 3 (mouse MCH1R).

- Preferably, the MCH-R chimeric protein comprises an MCH-R binding region characteristic of a human MCH-R along with transmembrane and intracellular  
20 domains characteristic of a non-human MCH-R. There are substantial amino acid differences between the N-terminus of the MCH-R present in humans and that present in other species such as mice. Such differences could result in, for example, the mouse MCH-R having different intrinsic properties and responsiveness to agonists and/or antagonists than the human MCH-R. The presence of a human MCH-R  
25 binding region provides for a "humanized" MCH-R chimeric receptor.

- The transmembrane and intracellular domains characteristic of a non-human MCH-R can be used in conjunction with a non-human host to provide a more naturally occurring environment for these regions. For example, an MCH-R chimeric having mouse transmembrane and intracellular domains are preferably used in murine  
30 cells lines or in transgenic mice.

MCH-R chimeric proteins may contain regions other than extracellular, transmembrane, and intracellular domains that do not substantially decrease the activity of the protein. Preferably, additional regions do not cause a decrease of more than about 25% of MCH-R activity as measured using one or more of the assays

described in the examples provided below. Examples of additional regions that may be present include fluorescent protein regions and linker regions.

In an embodiment of the present invention, the MCH-R chimeric protein comprises: (a) an MCH binding region characteristic of a first species and (b) a transmembrane and intracellular domain region characteristic of a second species joined directly, or through a linker, to the carboxy side of the MCH binding region. Preferably, the protein comprises, consists, or consists essentially of an MCH-R polypeptide having a sequence similarity of at least about 75%, at least 85%, or at least 95% with either SEQ. ID. NO. 4 (human short form/mouse species chimeric MCH1R) or SEQ. ID. NO. 5 (human long form/mouse species chimeric). Even more preferably, the protein comprises, consists essentially of, or consists of, SEQ. ID. NO. 4 or SEQ. ID. NO. 5.

Sequence similarity for polypeptides can be determined by BLAST. (Altschul *et al.*, 1997. *Nucleic Acids Res.* 25, 3389-3402, hereby incorporated by reference herein.) In an embodiment of the present invention, sequence similarity is determined using tBLASTn search program with the following parameters: MATRIX:BLOSUM62, PER RESIDUE GAP COST: 11, and Lambda ratio: 1.

Differences in naturally occurring amino acids are due to different R groups. An R group effects different properties of the amino acid such as physical size, charge, and hydrophobicity. Amino acids can be divided into different groups as follows: neutral and hydrophobic (alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, and methionine); neutral and polar (glycine, serine, threonine, tyrosine, cysteine, asparagine, and glutamine); basic (lysine, arginine, and histidine); and acidic (aspartic acid and glutamic acid).

Generally, in substituting different amino acids it is preferable to exchange amino acids having similar properties. Substituting different amino acids within a particular group, such as substituting valine for leucine, arginine for lysine, and asparagine for glutamine are good candidates for not causing a change in polypeptide functioning.

Changes outside of different amino acids groups can also be made. Preferably, such changes are made taking into account the position of the amino acid to be substituted in the polypeptide. For example, arginine can substitute more freely for nonpolar amino acids in the interior of a polypeptide than glutamate because of its long aliphatic side chain. (See, Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, Supplement 33 Appendix 1C.)

### MCH-R Fusion Proteins

MCH-R fusion proteins contain an MCH-R polypeptide region and a fluorescent protein region either directly joined together or joined together through a linker. These regions provide MCH-R activity and a marker for evaluating MCH-R dynamics.

An MCH-R polypeptide region provides functional MCH-R activity and includes naturally occurring MCH-R, chimeric MCH-R, and derivatives thereof. Preferred derivatives thereof have a sequence similarity of at least about 75%, at least about 85%, or at least about 95% to a naturally occurring MCH-R or a chimeric MCH-R described herein.

A fluorescent protein region contains a chromophore that fluoresces. Preferably, the fluorescent protein region is the green fluorescent protein of the jellyfish *Aequorea victoria* or a derivative thereof. Preferred derivatives have a sequence similarity of at least about 75%, at least about 85%, or at least about 95% to the *Aequorea victoria* green fluorescent protein (GFP). The *Aequorea victoria* green fluorescent protein and examples of derivatives thereof are described by Cormack *et al.*, 1996. *Gene* 17, 33-38; Yang *et al.*, 1996. *Nucleic Acids Research* 24, 4592-4593; Tsien *et al.*, U.S. Patent No. 5,625,048; Tsien *et al.*, U.S. Patent No. 5,777,079; and Cormack *et al.*, U.S. Patent No. 5,804,387 (each of which are hereby incorporated by reference herein).

In different embodiments the MCH-R polypeptide region comprises, consists essentially of, or consists of, a sequence selected from the group consisting of: SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3, SEQ. ID. NO. 4, and SEQ. ID. NO. 5; and the fluorescent polypeptide region comprises, consists essentially of, or consists of, an amino acid sequence selected from the group consisting of SEQ. ID. NO. 6 (GFP), SEQ. ID. NO. 7 (EGFP), SEQ. ID. NO. 8 (Emerald), SEQ. ID. NO. 9 (Topaz), and SEQ. ID. NO. 10 (W1b). EGFP, Emerald, Topaz, and W1b are derivatives of GFP.

The optionally present linker is a polypeptide region that is preferably from 1 to about 100 amino acids in length. In different embodiments the linker is up to 75, 50 or 25 amino acids in length.

Preferably, the MCH-R fusion protein comprises, consists essentially of, or consists of, the MCH-R polypeptide region and the fluorescent polypeptide region. More preferably, the protein comprises, consists essentially of, or consists of,

an amino acid sequence selected from the group consisting of: SEQ. ID. NO. 11 (mouse MCH1R-linker-EGFP), SEQ. ID. NO. 12 (mouse MCH1R/EGFP direct fusion), SEQ. ID. NO. 13 (human short form/mouse species chimeric MCH1R-linker-EGFP), or SEQ. ID. NO. 14 (human long form/mouse species chimeric MCH1R-linker-EGFP).

#### MCH-R Chimeric and Fusion Proteins Nucleic Acid and Expression

MCH-R chimeric and fusion proteins can be produced using techniques well known in the art. Preferably, such proteins are produced by recombinant expression inside a host cell by way of an expression vector or by way of nucleic acid integrated into the host genome. Examples of nucleic acid sequences encoding for MCH-R polypeptide regions, fluorescent protein regions, MCH-R chimeric proteins, and MCH-R fusion proteins are provided for by SEQ. ID. NOs. 15-29 (see Example 1, *infra*).

Starting with a particular amino acid sequence and the known degeneracy of the genetic code, a large number of different encoding nucleic acid sequences can be obtained. The degeneracy of the genetic code arises because almost all amino acids are encoded for by different combinations of nucleotide triplets or codons. The translation of a particular codon into a particular amino acid is well known in the art (*see, e.g.,* Lewin *GENES IV*, p. 119, Oxford University Press, 1990).

Amino acids are encoded for by codons as follows:

A=Ala=Alanine: codons GCA, GCC, GCG, GCU

C=Cys=Cysteine: codons UGC, UGU

D=Asp=Aspartic acid: codons GAC, GAU

E=Glu=Glutamic acid: codons GAA, GAG

F=Phe=Phenylalanine: codons UUC, UUU

G=Gly=Glycine: codons GGA, GGC, GGG, GGU

H=His=Histidine: codons CAC, CAU

I=Ile=Isoleucine: codons AUA, AUC, AUU

K=Lys=Lysine: codons AAA, AAG

L=Leu=Leucine: codons UUA, UUG, CUA, CUC, CUG, CUU

M=Met=Methionine: codon AUG

N=Asn=Asparagine: codons AAC, AAU

P=Pro=Proline: codons CCA, CCC, CCG, CCU

Q=Gln=Glutamine: codons CAA, CAG

R=Arg=Arginine: codons AGA, AGG, CGA, CGC, CGG, CGU

S=Ser=Serine: codons AGC, AGU, UCA, UCC, UCG, UCU

T=Thr=Threonine: codons ACA, ACC, ACG, ACU

V=Val=Valine: codons GUA, GUC, GUG, GUU

5 W=Trp=Tryptophan: codon UGG

Y=Tyr=Tyrosine: codons UAC, UAU

Examples of techniques for introducing nucleic acid into a cell and expressing the nucleic acid to produce protein are provided in references such as Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, and  
10 Sambrook, *et al.*, in *Molecular Cloning, A Laboratory Manual*, 2<sup>nd</sup> Edition, Cold Spring Harbor Laboratory Press, 1989.

An expression vector contains recombinant nucleic acid encoding for a polypeptide along with regulatory elements for proper transcription and processing. The recombinant nucleic acid contains two or more nucleic acid regions not naturally  
15 associated with each other. Exogenous regulatory elements such as an exogenous promoter can be useful for expressing recombinant nucleic acid in a particular host. Examples of expression vectors are cloning vectors, modified cloning vectors, specifically designed plasmids, and viruses.

Generally, the regulatory elements that are present in an expression  
20 vector include a transcriptional promoter, a ribosome binding site, a terminator, and an optionally present operator. Another preferred element is a polyadenylation signal providing for processing in eukaryotic cells. Preferably, an expression vector also contains an origin of replication for autonomous replication in a host cell, a selectable marker, a limited number of useful restriction enzyme sites, and a potential for high  
25 copy number.

Expression vectors providing suitable levels of polypeptide expression in different hosts are well known in the art. Mammalian expression vectors well known in the art include pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), pCI-neo (Promega) and .lambda.ZD35 (ATCC 37565). Bacterial expression vectors well known in the art include pET11a (Novagen), lambda gt11 (Invitrogen), pcDNAII (Invitrogen), and pKK223-3 (Pharmacia). Fungal cell expression vectors well known

in the art include pYES2 (Invitrogen) and Pichia expression vector (Invitrogen).  
Insect cell expression vectors well known in the art include Blue Bac III (Invitrogen).

Recombinant host cells may be prokaryotic or eukaryotic. Examples of recombinant host cells include the following: bacteria such as *E. coli*; fungal cells  
5 such as yeast; mammalian cells such as human, bovine, porcine, monkey, hamster, and rodent; and insect cells such as *Drosophila* and silkworm derived cell lines. Commercially available mammalian cell lines include L cells L-M(TK.sup.-) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651),  
10 CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

To enhance expression in a particular host it may be useful to modify the sequence to take into account codon usage of the host. Codon usage of different  
15 organisms are well known in the art. (See, Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, Supplement 33 Appendix 1C.)

Expression vectors may be introduced into host cells using standard techniques. Examples of such techniques include transformation, transfection, lipofection, protoplast fusion, and electroporation.

20 Nucleic acid encoding for a polypeptide can be expressed in a cell without the use of an expression vector employing, for example, synthetic mRNA or native mRNA. Additionally, mRNA can be translated in various cell-free systems such as wheat germ extracts and reticulocyte extracts, as well as in cell based systems, such as frog oocytes. Introduction of mRNA into cell based systems can be achieved,  
25 for example, by microinjection.

Techniques for producing transgenic animals are well known in the art. Examples of such techniques are provided for by *Teratocarcinomas and embryonic stem cells: a practical approach*. Ed. By E. J. Robertson, IRL Press Limited, Oxford, England (1987); and *Gene Targeting: a practical approach*. Ed. By A. L. Joyner,  
30 Oxford University Press Inc. New York, NY (1993).

#### G-Protein Coupled Receptor Assays

MCH-R is G-protein coupled receptor. Techniques for measuring different G-protein activities, such as Gi/o, Gs, and Gq are well known in the art.  
35 MCH-R activity is preferably assayed for by measuring either Gi/o or Gq.



Gi/o and Gs activity can be measured using techniques such as a melonaphore assay, measuring cAMP production, measuring inhibition of cAMP accumulation, and measuring binding of  $^{35}\text{S}$ -GTP. cAMP can be measured using different techniques such as radioimmunoassay and indirectly by cAMP responsive gene reporter proteins.

Gq activity can be measured using techniques such as those measuring intracellular  $\text{Ca}^{2+}$ . Examples of techniques well known in the art that can be employed to measure  $\text{Ca}^{2+}$  include the use of dyes such as Fura-2 and the use of  $\text{Ca}^{2+}$ -bioluminescent sensitive reporter proteins such as aequorin. An example of a cell line employing aequorin to measure G-protein activity is HEK293/aeq17. (Button *et al.*, 1993. *Cell Calcium* 14, 663-671, and Feighner *et al.*, 1999. *Science* 284, 2184-2188, both of which are hereby incorporated by reference herein.)

Functional assays can be performed using individual compounds or preparations containing different compounds. A preparation containing different compounds where one or more compounds affect MCH-R chimeric or fusion protein activity can be divided into smaller groups of compounds to identify the compound(s) affecting MCH-R chimeric or fusion protein activity. In an embodiment of the present invention a test preparation containing at least 10 compounds is used in a functional assay.

Functional assays can be performed using recombinantly produced MCH-R chimeric or fusion protein present in different environments. Such environments include, for example, cell extracts and purified cell extracts containing the MCH-R chimeric or fusion protein expressed from recombinant nucleic acid and an appropriate membrane for the polypeptide; and the use of a purified MCH-R chimeric or fusion protein produced by recombinant means that is introduced into a different environment suitable for measuring G-protein activity.

#### Fluorescent Protein Assays

Fluorescent protein joined to an MCH receptor can be employed to study different aspects of receptor dynamics including receptor sequestration, receptor densitization, and receptor localization. The fluorescent protein can be used in *in vitro* or *in vivo* systems.

*In vitro* applications of fluorescent proteins can be performed using techniques well known in the art. Examples of such techniques are provided by Barak *et al.*, 1997. *Mol Pharm.* 5, 177-184; Tarasova *et al.*, 1997. *J. Biol. Chem.* 272,

14817-14824; Lin *et al.*, 1998. *Mol. Cell. Endo.* 146, 27-37; Tarasova *et al.*, 1998. *J. Biol. Chem.* 273, 15883-15886; Kallal *et al.*, 1998. *J. Biol. Chem.* 273, 322-328; Groake *et al.*, 1999. *J. Biol. Chem.* 274, 23263-23269; Doherty *et al.*, 1999. *Biochem. J.* 341, 415-422; Brock *et al.*, 1999. *Proc. Natl. Acad. Sci. USA* 96, 10123-10128; Cornea *et al.*, 1999. *Endocrinology* 140, 4272-4280; and Lembo *et al.*, 1999. *Nat. Cell Biol.* 1, 267-271 (these references are not admitted to be prior art to the claimed invention).

*In vivo* applications of fluorescent proteins can be performed using techniques well known in the art. Examples of such techniques are provided by Mombaerts *et al.*, 1996. *Cell* 87, 675-686; Rodriquez *et al.*, 1999. *Cell* 97. 199-208; 10 Spergel *et al.*, 1999. *J. Neurosci.* 1, 2037-2050; and Zuo *et al.*, 1999. *Proc. Natl. Acad. Sci. USA* 96, 14100-14105 (these references are not admitted to be prior art to the claimed invention).

## 15 EXAMPLES

Examples are provided below to further illustrate different features and advantages of the present invention. The examples also illustrate useful methodology for practicing the invention. These examples do not limit the claimed invention.

## 20 Example 1:

Amino acid and nucleic acid sequence information for SEQ. ID. NOs. 1-29 are provided below. SEQ. ID. NOs. 1-29 include examples of polypeptide and encoding nucleic acid sequences for MCH-R polypeptide regions, fluorescent polypeptide regions, fusion proteins and chimeric proteins. In some cases the encoding nucleic acid is shown with additional nucleic acid upstream or downstream from an open reading frame.

**SEQ. ID. NO. 1: Human long form MCH1R**

MSVGAMKKGVGRAVGLGGSGCQATEEDPLPNCGACAPGQGGRRWRLPQP  
30 AWVEGSSARLWEQATGTGWMDEASLLPTGPNASNTSDGPDNLTSAGSPPR  
TGSISYINIMPSVFGTICLLGIIGNSTVIFAVVKKSKLHWCNNVPDIFIINLSVVD  
LLFLLGMPFMIHQLMGNGVWHFGETMCTLITAMDANSQFTSTYILTAMADR  
YLATVHPISSTKFRKPSVATLVICLLWALSFISITPVWLYARLIPFGGAVGCGI  
RLPNPDTDLYWFTLYOFFLAFALPFVTTAAAYVRILQRMSTSSVAPASQRSIRLR

TKRVTRTAIAICLVFFVCWAPYYVLQLTQLSISRPTLTFVYLYNAAISLGYANS  
CLNPFVYIVLCETFRKRLVLSVKPAAQGQLRAVSNAQTADEERTESKGT

**SEQ. ID. NO. 2: Human short form MCH1R**

5 MDLEASLLPTGPNASNTSDGPDNLTSAGSPRTGSGISYNIIMPVSFGTICLLGI  
NSTVIFAVVKKSKLHWCNNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGVWH  
FGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSVATLVI  
CLLWALSFSITPVWLYARLIPFPGGAVGCGIRLPNPDTDLWFTLYQFFLAFA  
LPFVVITAAYVRILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWAPY  
10 YVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLSV  
KPAAQGQLRAVSNAQTADEERTESKGT

**SEQ. ID. NO. 3: Mouse MCH1R**

MDLQASLLSTGPNASNISDGQDNFTLAGPPRTRSVSYNIIMPVSFGTICLLGI  
15 VGNSTVIFAVVKKSKLHWCSNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGV  
WHFGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSMAT  
LVICLLWALSFSITPVWLYARLIPFPGGAVGCGIRLPNPDTDLWFTLYQFFLA  
FALPFVVITAAYVKILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWA  
PYYVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLS  
20 VKPAAQGQLRTVSNAQTADEERTESKGT

**SEQ. ID. NO. 4: Human short form/mouse species chimeric MCH1R**

MDLEASLLPTGPNASNTSDGPDNLTSAGSPRTGSGISYNIIMPVSFGTICLLGI  
NSTVIFAVVKKSKLHWCNNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGVWH  
25 FGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSMATLVI  
CLLWALSFSITPVWLYARLIPFPGGAVGCGIRLPNPDTDLWFTLYQFFLAFA  
LPFVVITAAYVKILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWAPY  
YVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLSV  
KPAAQGQLRTVSNAQTADEERTESKGT

30

**SEQ. ID. NO. 5: Human long form/mouse species chimeric MCH1R**

MSVGAMKKGVGRAVGLGGSGCQATEEDPLPNCGACAPGQGRRWRLPQP  
AWVEGSSARLWEQATGTGWMDEASLLPTGPNASNTSDGPDNLTSAGSPR  
TGSISYNIIMPVSFGTICLLGI  
35 NSTVIFAVVKKSKLHWCNNVPDIFIINLSVVD  
LLFLLGMPFMIHQLMGNGVWHFGETMCTLITAMDANSQFTSTYILTAMAIDR

YLATVHPISSTKFRKPSMATLVICLLWALSFISITPVWLYARLIPFPGGAVGCGI  
 RLPNPD TDLYWFTLYQFFLAFALPFVVITAAYVKILQRMTSSVAPASQRSIRLR  
 TKRVTRTAIAICLVFFVCWAPYYVLQLTQLSISRPTLTFVYLYNAAISLGYANS  
 CLNPFVYTVLCETFRKRLVLSVKPAAQGGQLRTVSNAQTADERTESKGT

5

**SEQ. ID. NO. 6: GFP**

MSKGEELFTGVVPILVELDGDVNGHKFSVS GEGEGDATY GKLTLKFICTTGKL  
 PVPWPTLVTTFSYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGN  
 YKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQ  
 10 KNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKD  
 PNEKRDH MVLLFVTAAGITHGMDELYK

**SEQ. ID. NO. 7: EGFP**

MVSKGEELFTGVVPILVELDGDVNGHKFSVS GEGEGDATY GKLTLKFICTTGK  
 15 LPVPWPTLVTTLT YGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDG  
 NYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADK  
 QKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSK  
 DPNEKRDH MVLLFVTAAGITLGMDLYK

**20 SEQ. ID. NO. 8: Emerald**

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp  
 Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr  
 Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr  
 Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys  
 25 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe  
 Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr  
 Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly  
 His Lys Leu Glu Tyr Asn Tyr Asn Ser His Lys Val Tyr Ile Thr Ala Asp Lys Gln Lys  
 Asn Gly Ile Lys Val Asn Phe Lys Thr Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu  
 30 Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn  
 His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met  
 Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys

**SEQ. ID. NO. 9: Topaz**

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp  
 Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr  
 Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr  
 5 Leu Val Thr Thr Phe Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Arg  
 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe  
 Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr  
 Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly  
 His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys  
 10 Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu  
 Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn  
 His Tyr Leu Ser Tyr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met  
 Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys

**15 SEQ. ID. NO. 10: W1B**

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp  
 Gly Asp Val Asn Gly His Arg Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr  
 Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr  
 Leu Val Thr Thr Leu Thr Trp Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys  
 20 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe  
 Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr  
 Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly  
 His Lys Leu Glu Tyr Asn Tyr Ile Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys  
 Asn Gly Ile Lys Ala His Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu  
 25 Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn  
 His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met  
 Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys

**SEQ. ID. NO. 11: Mouse MCH1R-linker-EGFP**

30 MDLQASLLSTGPNASNISDGQDNFTLAGPPPRTRSVSYINIIMPSVFGTICLLGI  
 VGNSTVIFAVVKKSKLHWCSNVPDIFINLSVVDLLFLLGMPFMIHQLMGNGV  
 WHFGETMCTLITAMDANSQFTSTYILTAMADRYLATVHPISSTKFRKPSMAT  
 LVICLLWALSFSITPVWLYARLIPFGGAVGCGIRLPNPD TDLYWFTLYQFFLA  
 FALPFVVITAAYVKILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWA  
 35 PYYVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYTVLCETFRKRLVLS

VKPAAQGQLRTVSNAQTADEERTESKGTVDGTAGPGSIATMVSKGEELFTGV  
 VPILVELDGDVNGHKFSVS GEGEGDATYGKLT LKFICTTGKLPVPWPPTLVTTL  
 TYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRA EVKFE  
 GDTLVNRIELKGIDFKEDGNILGHKLEYNYN SHNVYIMADKQKNGIKVNFKIR  
 5 HNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDHMLV  
 LEFVTAAGITLGMDELYK

**SEQ. ID. NO. 12: Mouse MCH1R/EGFP direct fusion**

MDLQASLLSTGPNASNISDGQDNFTLAGPPPRTRSVSYNIIMPSVFGTICLLGI  
 10 VGNSTVIFAVVKKSKLHWCSNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGV  
 WHFGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSMAT  
 LVICLLWALS FISITPVWLYARLIPFGGAVGCGIRLPNPDTDLYWFTLYQFFLA  
 FALPFVVITAA YVKILQRMTSSVAPASQRSIRLRTKR VTRTAIAICLVFFVCWA  
 PYYVLQLTQLSISRPTLTFVYLYNAAISLG YANSCLNPFVYIVLCETFRKRLVLS  
 15 VKPAAQGQLRTVSNAQTADEERTESKGTMVSKGEELFTGVVPILVELDGDVN  
 GHKFSVS GEGEGDATYGKLT LKFICTTGKLPVPWPPTLVTTLTYGVQCFSRYP  
 HMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRA EVKFEGDTLVNRIELKGI  
 DFKEDGNILGHKLEYNYN SHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLAD  
 HYQQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDHMLVLEFVTAAGITLG  
 20 MDELYK

**SEQ. ID. NO. 13: Human short form/mouse species chimeric MCH1R-linker-EGFP**

MDLEASLLPTGPNASNTSDGPDNLTSAGSPPRTGSSISYNIIMPSVFGTICLLGIIG  
 25 NSTVIFAVVKKSKLHWCNNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGVWH  
 FGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSMATLVI  
 CLLWALS FISITPVWLYARLIPFGGAVGCGIRLPNPDTDLYWFTLYQFFLAFA  
 LPFVVITAA YVKILQRMTSSVAPASQRSIRLRTKR VTRTAIAICLVFFVCWAPY  
 YVLQLTQLSISRPTLTFVYLYNAAISLG YANSCLNPFVYIVLCETFRKRLVLSV  
 30 KPAAQGQLRTVSNAQTADEERTESKGTVDGTAGPGSIATMVSKGEELFTGVV  
 PILVELDGDVNGHKFSVS GEGEGDATYGKLT LKFICTTGKLPVPWPPTLVTTLT  
 YGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRA EVKFEG  
 DTLVNRIELKGIDFKEDGNILGHKLEYNYN SHNVYIMADKQKNGIKVNFKIRH  
 NIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDHMLVLL  
 35 EFVTAAGITLGMDELYK

**SEQ. ID. NO. 14: Human long form/mouse species chimeric MCH1R-linker-EGFP**

MSVGAMKKGVGRAVGLGGGSGCQATEEDPLPNCGACAPGQGRRWRLPQP  
5 AWEVGSSARLWEQATGTGWMDLEASLLPTGPNASNTSDGPDNLTSA GSPPR  
TGSISYINIIMPSVFGTICLLGIIGNSTVIFAVVKKSKLHWCNNVPDIFIINLSVVD  
LLFLLGMPFMIHQLMGNGVWHFGETMCTLITAMDANSQFTSTYILTAMAI DR  
YLATVHPISSTKFRKPSMATLVICLLWALSFISITPVWLYARLIPFPGA VCGGI  
RLPNPD TDLYWFTLYQFFLAFALPFVVITAA YVKILQRMTSSVAPASQRSIRLR  
10 TKRVTRTAIAICLVFFVCWAPYYVLQLTQLSISRPTLTFVYLYNAAISLGYANS  
CLNPFVYTVLCETFRKRLVLSVKPAAQGGQLRTVSNAQTADEERTESKGTVDGT  
AGPGSIATMVSKGEELFTGVVPILVELDGDVNGHKFSVS GEGEGDATY GKLT  
LKFICTTGKLPVPWPTLVTTLT YGVQCFSRYPDHMKQHDFFKSAMPEGYVQE  
RTIFFKDDGNYKTRA EVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSH  
15 NVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHY  
LSTQSALSKDPNEKRDH MVLLEFVTAAGITLGMD ELYK

**SEQ. ID. NO. 15: Human long form MCH1R cDNA**

ATGTCAGTGGGAGCCATGAAGAAGGGAGTGGGGAGGGCAGTTGGGCTTG  
20 GAGGCGGCAGCGGCTGCCAGGCTACGGAGGAAGACCCCCTTCCCAACTGC  
GGGGCTTGCGCTCCGGGACAAGGTGGCAGGCGCTGGAGGCTGCCGCAGC  
CTGCGTGGGTGGAGGGGAGCTCAGCTCGGTTGTGGGAGCAGGCGACCGG  
CACTGGCTGGATGGACCTGGAAGCCTCGCTGCTGCCCCACTGGTCCCAACG  
CCAGCAACACCTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCT  
25 CCTCGCACGGGGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTC  
GGCACCATCTGCCTCCTGGGCATCATCGGGA ACTCCACGGTCATCTTCGCG  
GTCGTGAAGAAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTT  
CATCATCAACCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTT  
CATGATCCACCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCA  
30 TGTGCACCCTCATCACGGCCATGGCATGCAATAGTCAGTTCACCAGCACC  
TACATCCTGACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCC  
ATCTCTTCCACGAAGTTCCGGAAGCCCTCTGTGGCCACCCTGGTGATCTGC  
CTCCTGTGGGGCCCTCTCCTTCATCAGCATACCCCTGTGTGGCTGTATGCC  
AGACTCATCCCCTTCCCAGGAGGTGCAGTGGGCTGCGGCATACGCCTGCC  
35 CAACCCAGACACTGACCTCTACTGGTTCACCCTGTACCAGTTTTTCTCCTGGC

CTTTGCCCTGCCTTTTGTGGTCATCACAGCCGCATACGTGAGGATCCTGCA  
GCGCATGACGTCCTCAGTGGCCCCCGCCTCCCAGCGCAGCATCCGGCTGC  
GGACAAAGAGGGTGACCCGCACAGCCATCGCCATCTGTCTGGTCTTCTTT  
GTGTGCTGGGCACCCTACTATGTGCTACAGCTGACCCAGTTGTCCATCAGC  
5 CGCCCGACCCTCACCTTTGTCTACTTATACAATGCGGCCATCAGCTTGGGC  
TATGCCAACAGCTGCCTCAACCCCTTTGTGTACATCGTGCTCTGTGAGACG  
TTCCGCAAACGCTTGGTCCTGTCTGGTGAAGCCTGCAGCCCAGGGGCAGCT  
TCGCGCTGTCAGCAACGCTCAGACGGCTGACGAGGAGAGGACAGAAAGC  
AAAGGCACCTGA

10

**SEQ. ID. NO. 16: Human short form MCH1R cDNA**

ATGGACCTGGAAGCCTCGCTGCTGCCCCACTGGTCCCAATGCCAGCAACAC  
CTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCTCCTCGCACGG  
GGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTCGGCACCATCT  
15 GCCTCCTGGGCATCATCGGGAACCTCCACGGTCATCTTCGCGGTCTGTGAAG  
AAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTTCATCATCAA  
CCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTTCATGATCCA  
CCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCATGTGCACCC  
TCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACCTACATCCTG  
20 ACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCCATCTCTTCC  
ACGAAGTTCCGGAAGCCCTCTGTGGCCACCCTGGTGATCTGCCTCCTGTGG  
GCCCTCTCCTTCATCAGCATCACCCCTGTGTGGCTGTATGCCAGACTCATC  
CCCTTCCCAGGAGGTGCAGTGGGCTGCGGCATACGCCTGCCCAACCCAGA  
CACTGACCTCTACTGGTTCACCCTGTACCAGTTTTTCCTGGCCTTTGCCCTG  
25 CCTTTTGTGGTCATCACAGCCGCATACGTGAGGATCCTGCAGCGCATGAC  
GTCCTCAGTGGCCCCCGCCTCCCAGCGCAGCATCCGGCTGCGGACAAAGA  
GGGTGACCCGCACAGCCATCGCCATCTGTCTGGTCTTCTTTGTGTGCTGGG  
CACCCCTACTATGTGCTACAGCTGACCCAGTTGTCCATCAGCCGCCCCGACCC  
TCACCTTTGTCTACTTATACAATGCGGCCATCAGCTTGGGCTATGCCAACA  
30 GCTGCCTCAACCCCTTTGTGTACATCGTGCTCTGTGAGACGTTCCGCAAAC  
GCTTGGTCCTGTCTGGTGAAGCCTGCAGCCCAGGGGCAGCTTCGCGCTGTC  
AGCAACGCTCAGACGGCTGACGAGGAGAGGACAGAAAGCAAAGGCACCT  
GA



**SEQ. ID. NO. 17: Mouse MCH1R cDNA**

Nucleic acid sequence start and stop codons are highlighted:

GGCGGTAGAGGAAGACCCTTTTCTGGACTGCGGGGCTCAAGCTCCGGACA  
AGGCGGTGGAGGGCGCTGGAGGCTGCCGCAGCCTGCGTGGGTGGACGGG  
5 CGCTCCACTCCAGGGAGCAGGCGACCTGCACCGGCTGCATGGATCTGCAA  
GCCTCGTTGCTGTCCACTGGCCCCAATGCCAGCAACATCTCCGATGGCCA  
GGATAATTTACATTGGCGGGGCCACCTCCTCGCACAAGGAGTGTCTCCT  
ACATCAACATCATCATGCCTTCAGTGTTTGGTACCATCTGTCTCCTGGGCA  
TTGTGGGAAACTCCACAGTCATTTTTGCCGTGGTGAAGAAATCCAAGCTG  
10 CACTGGTGCAGCAACGTCCCTGACATCTTCATCATCAACCTCTCTGTGGTG  
GATCTGCTTTTCCTGCTGGGCATGCCTTTCATGATCCACCAGCTCATGGGT  
AATGGTGTCTGGCACTTTGGGGAAACCATGTGCACCCTCATCACAGCCAT  
GGACGCCAACAGTCAGTTCACCAGCACCTACATCCTGACTGCTATGGCCA  
TTGACCGCTACTTGGCCACCGTCCATCCCATCTCCTCCACCAAGTTCCGGA  
15 AGCCCTCCATGGCCACCCTGGTGATCTGCCTCCTGTGGGCTCTCTCGTTCA  
TTAGCATCACTCCTGTGTGGCTCTATGCCAGGCTTATCCCCTTCCCAGGGG  
GTGCTGTGGGCTGTGGCATCCGCCTACCAAACCCAGATACTGATCTTTACT  
GGTTCACTCTGTATCAGTTTTTCTGTCCTTCGCCCTTCGGTTTGTGGTCAT  
CACTGCTGCGTACGTGAAAATACTACAGCGCATGACGTCTTCGGTGGCCC  
20 CAGCCTCTCAACGCAGCATCCGGCTTCGGACAAAGAGGGTGACCCGCACA  
GCCATTGCCATCTGTCTGGTCTTCTTTGTGTGCTGGGCGCCCTACTACGTG  
CTGCAGCTGACCCAGTTGTCCATCAGCCGCCCGACCCTCACATTCTGTCTAC  
CTGTACAATGCGGCCATCAGCTTGGGCTATGCCAACAGCTGCCTCAATCC  
CTTTGTGTACATAGTACTCTGTGAGACCTTTTCGAAAACGCTTGGTGTCTGTC  
25 GGTGAAGCCCGCGGCCAGGGGCAGCTTCGCACGGTCAGCAATGCTCAGA  
CAGCTGACGAGGAGAGGACAGAAAGCAAAGGCACCTGACAATCCCCCCC  
GGTCACCTCCAAGTCAGGTCACCGCATCAAACCATGGGGAGAGATACTGA  
GATAAACCCGGGGCTACCCTGGGAGGATGCAGAAGCTGGAGGCTGGGGG  
CTTGTAGCAAACCACATTCCACGGGGGCCACAAATTGCTAGGGAGGCTTG  
30 CAGCCTGGTTTGGGGGGGAAGCCTCAGACTGCAGGGATCCCCTTGACAGA  
ATAGAAGCGGAGCAAGAAGGAAAGGGTGGTTTACTGGTTCTCGGGGTCT  
GTATCTGTTGGCTCGCATATATCTTTCTCTCAAGGGAAGAAGGCGGAGGT  
GCCTAGCTGGGTTCCTTTAAACTAGGCAGGGCTAGGATCTGAGCAGCTA  
GGGCTCTACTGTGAGACTGGGCAAGCCGAGCGTTCCTCCCATCTCTCATT  
35 GGTGTTGATAGAAGGCAGTCTTTCTCCCAAGCTGGTGGATCTCCTGAAGC

ACGCTGCCTGGGCTCCAGCATCCTGTGCGGATTTACGTTCTCTTTAGGGG  
ATGCATGTTGACACTGGGGTGTGGGCTCTGAGCCCACAGGAGTTTAAAAA  
ACCAAAAGAGCTCAGAGTGTGAGAGAGACCCAATCACCGAGAATGACA  
AGGCAACCTGGGGTGGATGTGGATCTTGAACTAATAAAAAGGGGTTTTC  
5 ACAGTGACAGCGACATTCTCTTCATAGGGCACAGCTGTCAGTCTATGGCT  
GATCCAGAGCGAGCATCCATGAATTCTGCATGTGCAGGGGTCACTCTAAT  
ACCTGATATGTTGGCATCATCTTTGTGCTTGAGCCTTCNCTCCCAAATGG  
GAATGAAATAAAGGCAAATTCCNCCCCCCCCCAAAAAGGGGNAAAAAA  
AAAAAAAAAAAAAAAAAAAAA

10

**SEQ. ID. NO. 18: Mouse MCH1R genomic DNA**

Nucleic acid sequence start and stop codons, as well as intron borders, are highlighted:

GGCGGTAGAGGAAGACCCTTTTCTGGACTGCGGGGCTCAAGCTCCGGACA  
15 AGGCGGTGGAGGGCGCTGGAGGCTGCCGCAGCCTGCGTGGGTGGACGGG  
CGTCCACTCCAGGGAGCAGGCGACCTGCACCGGCTGCATGGATCTGCAA  
GCCTCGTTGCTGTCCACTGGCCCCAATGCCAGCAACATCTCCGATGGCCA  
GGATAATTTACATTGGCGGGTGAGTCGAGTTGGAGTCCTCCCTCCTCCG  
GGATGGGTGTGGAAAATGGGAAGGTTTACCTCCCAAGCCAAACTGCCTG  
20 GGAACTTTATCTTACAGTTCTTGGTGATAAGATCTGCAGTCGGCTTTGCC  
TGAAGAGGAAGAGGAGAGGAGGGGACACCAGCTAGGACAGAAGGGGCA  
GGGAGGAATAGAGATGGGGCAGAGGCACATTTAGAAACAACAAGGGTTG  
GTGACAAGACGTGAGGCAGGCTTGAGGGGAAAGCTTGCTGATGAGTCCCA  
AATATGCTTTGCAGGGGGGGGGGGGGGAATCAAGGCTGGAGAAGCAA  
25 GCAAGCAAGACAGCAAGACAGCGGGCGGGTAGTATGTGGGAGCCAGCAG  
AAGCGCTTTGATTCACCGCTATCCTGGGCTCAATCCTCTGGCCTCGCACTG  
GGGAAATGGGGTCTGAGTGGTCCTTGCTGTCTTCTGGCAAAGGCTGCTGG  
GAGCAAAAGACTTCACAGGGCGTGAGAGGATTAACCTTTTCTGGTGAATTA  
AGCTTCTTGACATTTGCAGAACGTCAATGCCTTAAAATTCTAGCTCTGAAG  
30 GAGAAGGGAATGAAGGGGAAAGAGGGAAGGTTGGTGTGGAGAAATTCCC  
AAGCTTCTGGGGTGTAACACAGCTCCAGTCCCTACCCTATTGGGAAAGCC  
CAGACTCAGGAGACATGGTCCAAGGAAATCCCTGACAGAAAACCGGGAG  
AGGGCAGGGCTGTGGAGCCTGAAACACACCCACACCCATGGTGACAGTC  
ACTTCTCACATATGCCTAGGAACCTATCTGAAACCTTTGGCCATCTCTCTC  
35 TGAAGAGATGAGGCTGCAAATACACACACACACACACACACACACAC

ACACACACACACACACACACACACACACACACAAATGTCCTTCAAGCC  
TTTTTGACAAGGTTTTCTGGTGGATCCCGGGGATATGAAGTTGTTCTCAGC  
AGATATCTGGGAGTCTTGACTCCTGGCCCTCTGAGTAAATGGATGAAGCG  
AAGAAGAATGGGGTCCTCTGAGTAACAGGTGGATCTAGAAAATCCTATAG  
5 GAGTCACCAGGGCACGGTGGAGGAGGGTAAGGTACAGAACTAACAATAG  
CCCGAGAAGGGGAAACAGCAGGAGATGATTCCAGAGACGTAGTGACCCC  
AAGCTGCAAGGGAAAGCATGAGGGGCCAGCAGGAAGGCCGACATGGCAG  
GTTGTCAGCTTCTAGATCGGAAGGCGGGTCACACTTGCTCTTTCTATCCTC  
AGGGCCACCTCCTCGCACAAGGAGTGTCTCCTACATCAACATCATCATGC  
10 CTTCAGTGTTTGGTACCATCTGTCTCCTGGGCATTGTGGGAAACTCCACAG  
TCATTTTTGCCGTGGTGAAGAAATCCAAGCTGCACTGGTGCAGCAACGTC  
CCTGACATCTTCATCATCAACCTCTCTGTGGTGGATCTGCTTTTCCTGCTGG  
GCATGCCTTTTCATGATCCACCAGCTCATGGGTAAATGGTGTCTGGCACTTTG  
GGGAAACCATGTGCACCCTCATCACAGCCATGGACGCCAACAGTCAGTTC  
15 ACCAGCACCTACATCCTGACTGCTATGGCCATTGACCGCTACTTGGCCACC  
GTCCATCCCATCTCCTCCACCAAGTTCCGGAAGCCCTCCATGGCCACCCTG  
GTGATCTGCCTCCTGTGGGCTCTCTCGTTTATTAGCATCACTCCTGTGTGG  
CTCTATGCCAGGCTTATCCCCTTCCCAGGGGGTGCTGTGGGCTGTGGCATC  
CGCCTACCAAACCCAGATACTGATCTTTACTGGTTCACTCTGTATCAGTTT  
20 TTCCTGGCCTTCGCCCTTCCGTTTGTGGTCATCACTGCTGCGTACGTGAAA  
ATACTACAGCGCATGACGTCTTCGGTGGCCCCAGCCTCTCAACGCAGCAT  
CCGGCTTCGGACAAAGAGGGTGACCCGCACAGCCATTGCCATCTGTCTGG  
TCTTCTTTGTGTGCTGGGCGCCCTACTACGTGCTGCAGCTGACCCAGTTGT  
CCATCAGCCGCCCCGACCCTCACATTCTGTCTACCTGTACAATGCGGCCATCA  
25 GCTTGGGCTATGCCAACAGCTGCCTCAATCCCTTTGTGTACATAGTACTCT  
GTGAGACCTTTCGAAAACGCTTGGTGTGCTGTGGTGAAGCCCGCGGCCAG  
GGGCAGCTTCGCACGGTCAGCAATGCTCAGACAGCTGACGAGGAGAGGA  
CAGAAAGCAAAGGCACCTGACAATCCCCCCCCGGTCACCTCCAAGTCAGGT  
CACCGCATCAAACCATGGGGAGAGATACTGAGATAAACCCGGGGCTACC  
30 CTGGGAGGATGCAGAAGCTGGAGGCTGGGGGCTTGTAGCAAACCACATTC  
CACGGGGCCCCACAAATTGCTAGGGAGGCTTGCAGCCTGGTTTGGGGGGGA  
AGCCTCAGACTGCAGGGATCCCCCTGACAGAATAGAAGCGGAGCAAGAA  
GGAAAGGGTGGTTTACTGGTTCTCGGGGTCTGTATCTGTTGGCTCGCATA  
TATCTTTCTCTCAAGGGAAGAAGGCGGAGGTGCCTAGCTGGGTTCTTTA  
35 AAAGTAGGCAGGGCTAGGATCTGAGCAGCTAGGGCTCTACTGTGAGACTG

GGCAAGCCGAGCGTTCCCTCCCATCTCTCATTGGTGTTGATAGAAGGCAG  
TCTTTCTCCCAAGCTGGTGGATCTCCTGAAGCACGCTGCCTGGGCTCCAGC  
ATCCTGTGCGGATTTACGTTCTCTTTAGGGGATGCATGTTGACACTGGGG  
TGTGGGCTCTGAGCCCAAGGAGTTAAAAAACCAAAAGAGCTCAGAGTG  
5 TCGAGAGAGACCCAATCACCGAGAATGACAAGGCAACCTGGGGTGGATG  
TGGATCTTGAACTAATAAAAAGGGGTTTTTCACAGTGACAGCGACATTCT  
CTTCATAGGGCACAGCTGTCAGTCTATGGCTGATCCAGAGCGAGCATCCA  
TGAATTCTGCATGTGCAGGGGTCACTCTAATACCTGATATGTTGGCATCAT  
CTTTGTGCTTGAGCCTTCNCTCCCAAATGGGAATGAAATAAAGGCAAAT  
10 TCCCNCCCCCCCCAAAAAGGGGNAAAAAAAAAAAAAAAAAAAAAAAAAA  
AA

**SEQ. ID. NO. 19: Human short form/mouse species chimeric MCH1R**

ATGGACCTGGAAGCCTCGCTGCTGCCCACTGGTCCCAATGCCAGCAACAC  
15 CTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCTCCTCGCACGG  
GGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTCGGCACCATCT  
GCCTCCTGGGCATCATCGGGAACCTCCACGGTCATCTTCGCGGTCTGTGAAG  
AAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTTCATCATCAA  
CCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTTCATGATCCA  
20 CCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCATGTGCACCC  
TCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACCTACATCCTG  
ACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCCATCTCTTCC  
ACGAAGTTCCGGAAGCCCTCCATGGCCACCCTGGTGATCTGCCTCCTGTG  
GGCTCTCTCGTTTCATTAGCATCACTCCTGTGTGGCTCTATGCCAGGCTTAT  
25 CCCCTTCCCAGGGGGTGCTGTGGGCTGTGGCATCCGCCTACCAAACCCAG  
ATACTGATCTTTACTGGTTCACTCTGTATCAGTTTTTCTGGCCTTCGCCCT  
TCCGTTTGTGGTCATCACTGCTGCGTACGTGAAAATACTACAGCGCATGAC  
GTCTTCGGTGGCCCCAGCCTCTCAACGCAGCATCCGGCTTCGGACAAAGA  
GGGTGACCCGCACAGCCATTGCCATCTGTCTGGTCTTCTTTGTGTGCTGGG  
30 CGCCCTACTACGTGCTGCAGCTGACCCAGTTGTCCATCAGCCGCCCGACC  
CTCACATTCGTCTACCTGTACAATGCGGCCATCAGCTTGGGCTATGCCAAC  
AGCTGCCTCAATCCCTTTGTGTACATAGTACTCTGTGAGACCTTTCGAAAA  
CGCTTGGTGCTGTGCGTGAAGCCCGCGGCCAGGGGCAGCTTCGCACGGT  
CAGCAATGCTCAGACAGCTGACGAGGAGAGGACAGAAAGCAAAGGCACC  
35 TGA

**SEQ. ID. NO. 20: Human long form/mouse species chimeric MCH1R**

ATGTCAGTGGGAGCCATGAAGAAGGGAGTGGGGAGGGCAGTTGGGCTTG  
GAGGCGGCAGCGGCTGCCAGGCTACGGAGGAAGACCCCCTTCCCAACTGC  
5 GGGGCTTGCGCTCCGGGACAAGGTGGCAGGCGCTGGAGGCTGCCGCAGC  
CTGCGTGGGTGGAGGGGAGCTCAGCTCGGTTGTGGGAGCAGGCGACCGG  
CACTGGCTGGATGGACCTGGAAGCCTCGCTGCTGCCCCTGGTCCCAACG  
CCAGCAACACCTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCT  
CCTCGCACGGGGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTC  
10 GGCACCATCTGCCTCCTGGGCATCATCGGGAAGTCCACGGTCATCTTCGCG  
GTCGTGAAGAAGTCCAAGCTGCACTGGTGAACAACGTCCCCGACATCTT  
CATCATCAACCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTT  
CATGATCCACCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCA  
TGTGCACCCTCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACC  
15 TACATCCTGACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCC  
ATCTCTTCCACGAAGTTCGGGAAGCCCTCCATGGCCACCCTGGTGATCTGC  
CTCCTGTGGGCTCTCTCGTTCATTAGCATCACTCCTGTGTGGCTCTATGCC  
AGGCTTATCCCCTTCCCAGGGGGTGTGTGGGCTGTGGCATCCGCCTACCA  
AACCCAGATACTGATCTTTACTGGTTCCTGTATCAGTTTTCTGGCCT  
20 TCGCCCTTCCGTTTGTGGTCATCACTGCTGCGTACGTGAAAATACTACAGC  
GCATGACGTCTTCGGTGGCCCCAGCCTCTCAACGCAGCATCCGGCTTCGG  
ACAAAGAGGGTGACCCGCACAGCCATTGCCATCTGTCTGGTCTTCTTTGTG  
TGCTGGGCGCCCTACTACGTGCTGCAGCTGACCCAGTTGTCCATCAGCCGC  
CCGACCCTCACATTCGTCTACCTGTACAATGCGGCCATCAGCTTGGGCTAT  
25 GCCAACAGCTGCCTCAATCCCTTTGTGTACATAGTACTCTGTGAGACCTTT  
CGAAAACGCTTGGTGCTGTTCGGTGAAGCCCGCGGCCAGGGGCAGCTTCG  
CACGGTCAGCAATGCTCAGACAGCTGACGAGGAGAGGACAGAAAGCAAA  
GGCACCTGA

**30 SEQ. ID. NO. 21: *Aequorea victoria* Green Fluorescent Protein (GFP) cDNA**

Nucleic acid sequence start and stop codons are highlighted:

TACACACGAATAAAAGATAACAAAGATGAGTAAAGGAGAAGAACTTTTC  
ACTGGAGTTGTCCCAATTCTTGTGAATTAGATGGTGATGTTAATGGGCAC  
AAATTTTCTGTCTAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAAC  
35 TACCCTTAAATTTATTTGCACTACTGGAAAACCTGTTCCATGGCCAAC

ACTTGTCACTACTTTCTCTTATGGTGGTCAATGCTTTTCAAGATACCCAGAT  
 CATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCCGAAGGTTATGT  
 ACAGGAAAGAACTATATTTTTCAAGATGACGGGAACTACAAGACACGTG  
 CTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAA  
 5 GGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATA  
 CAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATG  
 GAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTT  
 CAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGT  
 CCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTGAAAGA  
 10 TCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAAACAGCTG  
 CTGGGATTACACATGGCATGGATGAACTATACAAATAAATGTCCAGACTT  
 CCAATTGACACTAAAGTGTCCGAACAATTACTAAAATCTCAGGGTTCCTG  
 GTTAAATTCAGGCTGAGATATTATTTATATATTTATAGATTCATTAAAT  
 GTATGAATAATTTATTGATGTTATTGATAGAGGTTATTTTCTTATTAAACA  
 15 GGCTACTTGGAGTGTATTCTTAATTCTATATTAATTACAATTTGATTTGACT  
 TGCTCAA

**SEQ. ID. NO. 22: EGFP + Linker**

Nucleic acid sequence start and stop codons are highlighted and a 12 amino acid

20 linker sequence is denoted in lower case:

gtcgacggtaccgcgggcccgatccatcgccaccATGGTGAGCAAGGGCGAGGAGCTGTT  
 CACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCC  
 ACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAA  
 GCTGACCCTGAAGTTCATCTGCACCACCGCAAGCTGCCCGTGCCCTGGC  
 25 CCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTAC  
 CCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCCGAAGG  
 CTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGA  
 CCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAG  
 CTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGC  
 30 TGGAGTACAACACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAG  
 AAGAACGGCATCAAGGTGAACCTTCAAGATCCGCCACAACATCGAGGACG  
 GCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGAC  
 GGCCCCGTGCTGCTGCCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT  
 GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCCG

TGACCGCCGCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGC  
GGCCGC

**SEQ. ID. NO. 23: Emerald**

5 ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGT  
CGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAG  
GGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCAC  
CACCGGCAAGCTGCCCCGTGCCCTGGCCACCCTCGTGACCACCTTGACCT  
ACGGCGTGCAAGTCTTCGCCCCGCTACCCCGACCACATGAAGCAGCACGAC  
10 TTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTC  
TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGG  
GCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAG  
GACGGCAACATCCTGGGGCACAAGCTGGAGTACAACACTACAACAGCCACA  
AGGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTC  
15 AAGACCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACT  
ACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAAC  
CACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCG  
CGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCGGGATCACTCTCG  
GCATGGACGAGCTGTACAAGTAA

20

**SEQ. ID. NO. 24: Topaz**

ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGT  
CGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAG  
GGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCAC  
25 CACCGGCAAGCTGCCCCGTGCCCTGGCCACCCTCGTGACCACCTTCGGCT  
ACGGCGTGCAAGTCTTCGCCCCGCTACCCCGACCACATGCGCCAGCACGAC  
TTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTC  
TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGG  
GCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAG  
30 GACGGCAACATCCTGGGGCACAAGCTGGAGTACAACACTACAACAGCCACA  
ACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTC  
AAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTA  
CCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACC  
ACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGC

GATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGG  
CATGGACGAGCTGTACAAGTAA

**SEQ. ID. NO. 25: W1B**

5 ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGT  
CGAGCTGGACGGCGACGTAAACGGCCACAGGTTACGCGTGTCCGGCGAG  
GGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCAC  
CACCGGCAAGCTGCCCCGTGCCCTGGCCCCACCCTCGTGACCACCCTGACCT  
GGGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGAC  
10 TTCTTCAAGTCCGCCATGCCCCAAGGCTACGTCCAGGAGCGTACCATCTTC  
TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGG  
GCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAG  
GACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACATCAGCCACA  
ACGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAGGCCCACTTC  
15 AAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTA  
CCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACC  
ACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGC  
GATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGG  
CATGGACGAGCTGTACAAGTAA

20

**SEQ. ID. NO. 26: Mouse MCH1R-linker-EGFP**

Nucleic acid sequence start codon and start and stop codons for mouse MCH1R and EGFP, respectively, as well as intron borders, are highlighted and a 12 amino acid linker sequence is denoted in lower case:

25 ATGGATCTGCAAGCCTCGTTGCTGTCCACTGGCCCCAATGCCAGCAACAT  
CTCCGATGGCCAGGATAATTTACATTGGCGGGTGAGTCGAGTTGGAGTC  
CTCCCTCCTCCGGGATGGGTGTGGAAAATGGGAAGGTTTCACCTCCCAAG  
CCAAACTGCCTGGGAACTTTATCTTACAGTTCTTGGTGATAAGATCTGCA  
GTCGGCTTTGCCTGAAGAGGAAGAGGAGAGGAGGGGACACCAGCTAGGA  
30 CAGAAGGGGCAGGGAGGAATAGAGATGGGGCAGAGGCACATTTAGAAAC  
AACAAGGGTTGGTGACAAGACGTGAGGCAGGCTTGAGGGGAAAGCTTGC  
TGATGAGTCCCAAATATGCTTTGCAGGGGGGGGGGGGGGGAATCAAGG  
CTGGAGAAGCAAGCAAGCAAGACAGCAAGACAGCGGGCGGGTAGTATGT  
GGGAGCCAGCAGAAGCGCTTTGATTACCGCTATCCTGGGCTCAATCCTC  
35 TGGCCTCGCACTGGGGAAATGGGGTCTGAGTGGTCCTTGCTGTCTTCTGGC



AAAGGCTGCTGGGAGCAAAAGACTTCACAGGGCGTGAGAGGATTAACCTTT  
TCTGGTGAATTAAGCTTCTTGACATTTGCAGAACGTCAATGCCTTAAAT  
CTAGCTCTGAAGGAGAAGGGAATGAAGGGGAAAGAGGGAAGGTTGGTGT  
GGAGAAATTCCCAAGCTTCTGGGGTGTAACACAGCTCCAGTCCCTACCT  
5 ATTGGGAAAGCCCAGACTCAGGAGACATGGTCCAAGGAAATCCCTGACA  
GAAAACCGGGAGAGGGCAGGGCTGTGGAGCCTGAAACACACCCACACC  
CATGGTGACAGTCACTTCTCACATATGCCTAGGAACCTATCTGAAACCTTT  
GGCCATCTCTCTCTGAAAAGATGAGGCTGCAAATACACACACACACAC  
ACAAA  
10 TGTCTTCAAGCCTTTTTGACAAGGTTTTCTGGTGGATCCCGGGGATATGA  
AGTTGTTCTCAGCAGATATCTGGGAGTCTTGACTCCTGGCCCTCTGAGTAA  
ATGGATGAAGCGAAGAAGAATGGGGTCCTCTGAGTAACAGGTGGATCTA  
GAAATCCTATAGGAGTCACCAGGGCACGGTGGAGGAGGGTAAGGTACA  
GAACTAACAATAGCCCGAGAAGGGGAAACAGCAGGAGATGATTCCAGAG  
15 ACGTAGTGACCCCAAGCTGCAAGGGAAAGCATGAGGGGCCAGCAGGAAG  
GCCGACATGGCAGGTTGTCAGCTTCTAGATCGGAAGGCGGGTCACACTTG  
CTCTTTCTATCCTCAGGGCCACCTCCTCGCACAAGGAGTGTCTCCTACATC  
AACATCATCATGCCTTCAGTGTTTGGTACCATCTGTCTCCTGGGCATTGTG  
GGAAACTCCACAGTCATTTTTGCCGTGGTGAAGAAATCCAAGCTGCACTG  
20 GTGCAGCAACGTCCCTGACATCTTCATCATCAACCTCTCTGTGGTGGATCT  
GCTTTTCTGCTGGGCATGCCTTTCATGATCCACCAGCTCATGGGTAATGG  
TGTCTGGCACTTTGGGGAAACCATGTGCACCCTCATCACAGCCATGGACG  
CCAACAGTCAGTTCACCAGCACCTACATCCTGACTGCTATGGCCATTGACC  
GCTACTTGGCCACCGTCCATCCCATCTCCTCCACCAAGTTCCGGAAGCCCT  
25 CCATGGCCACCCTGGTGATCTGCCTCCTGTGGGCTCTCTCGTTTATTAGCA  
TCACTCCTGTGTGGCTCTATGCCAGGCTTATCCCCTTCCCAGGGGGTGCTG  
TGGGCTGTGGCATCCGCCTACCAAACCCAGATACTGATCTTTACTGGTTCA  
CTCTGTATCAGTTTTTCTGGCCTTCGCCCTTCCGTTTGTGGTCATCACTGC  
TGCGTACGTGAAAATACTACAGCGCATGACGTCTTCGGTGGCCCCAGCCT  
30 CTCAACGCAGCATCCGGCTTCGGACAAAGAGGGTGACCCGCACAGCCATT  
GCCATCTGTCTGGTCTTCTTTGTGTGCTGGGCGCCCTACTACGTGCTGCAG  
CTGACCCAGTTGTCCATCAGCCGCCGACCCTCACATTCGTCTACCTGTAC  
AATGCGGCCATCAGCTTGGGCTATGCCAACAGCTGCCTCAATCCCTTTGTG  
TACATAGTACTCTGTGAGACCTTTCGAAAACGCTTGGTGCTGTCCGTGAA  
35 GCCCGCGGCCAGGGGCAGCTTCGCACGGTCAGCAATGCTCAGACAGCTG

ACGAGGAGAGGACAGAAAGCAAAGGCACCgtcgacgggtaccgcgggcccggtatccatcg  
 ccaccATGGTGAGCAAGGGCGAGGAGCTGTTACACGGGGTGGTGCCCATCC  
 TGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGC  
 GAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTG  
 5 CACCACCGGCAAGCTGCCCCGTGCCCTGGCCCCACCCTCGTGACCACCCTGA  
 CCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCAC  
 GACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCAT  
 CTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCG  
 AGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAG  
 10 GAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACACTACAACAGCC  
 ACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAA  
 CTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACC  
 ACTACCAGCAGAACACCCCCATCGGCGACGGCCCCCGTGCTGCTGCCCGAC  
 AACCCTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAA  
 15 GCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCCGCGGGATCACTC  
 TCGGCATGGACGAGCTGTACAAGTAA

**SEQ. ID. NO. 27: Mouse MCH1R/EGFP direct fusion**

Nucleic acid sequence start codon and start and stop codons for mouse MCH1R and  
 20 EGFP, respectively, as well as intron borders, are highlighted:  
 ATGGATCTGCAAGCCTCGTTGCTGTCCACTGGCCCCAATGCCAGCAACAT  
 CTCCGATGGCCAGGATAATTTACATTGGCGGGTGAGTCGAGTTGGAGTC  
 CTCCCTCCTCCGGGATGGGTGTGGAATAATGGGAAGGTTTACCTCCCAAG  
 CCAAAGTGCCTGGGAACTTTATCTTACAGTTCTTGGTGATAAGATCTGCA  
 25 GTCGGCTTTGCCTGAAGAGGAAGAGGAGAGGAGGGGACACCAGCTAGGA  
 CAGAAGGGGCAGGGAGGAATAGAGATGGGGCAGAGGCACATTTAGAAAC  
 ACAAAGGGTTGGTGACAAGACGTGAGGCAGGCTTGAGGGGAAAGCTTGC  
 TGATGAGTCCCAAATATGCTTTGCAGGGGGGGGGGGGGGGGAATCAAGG  
 CTGGAGAAGCAAGCAAGCAAGACAGCAAGACAGCGGGCGGGTAGTATGT  
 30 GGGAGCCAGCAGAAGCGCTTTGATTACCGCTATCCTGGGCTCAATCCTC  
 TGGCCTCGCACTGGGGAAATGGGGTCTGAGTGGTCCTTGCTGTCTTCTGGC  
 AAAGGCTGCTGGGAGCAAAAGACTTCACAGGGCGTGAGAGGATTAACCTT  
 TCTGGTGAATTAAGCTTCTTGACATTTGCAGAACGTCAATGCCTTAAATT  
 CTAGCTCTGAAGGAGAAGGGAATGAAGGGGAAAGAGGGAAGGTTGGTGT  
 35 GGAGAAATTCCCAAGCTTCTGGGGTGTAACACAGCTCCAGTCCCTACCTC

ATTGGGAAAGCCCAGACTCAGGAGACATGGTCCAAGGAAATCCCTGACA  
GAAAACCGGGAGAGGGCAGGGCTGTGGAGCCTGAAACACACCCCACACC  
CATGGTGACAGTCACTTCTCACATATGCCTAGGAACCTATCTGAAACCTTT  
GGCCATCTCTCTCTGAAAAGATGAGGCTGCAAATACACACACACACACAC  
5 ACAAA  
TGTCCTTCAAGCCTTTTTGACAAGGTTTTCTGGTGGATCCCGGGGATATGA  
AGTTGTTCTCAGCAGATATCTGGGAGTCTTGACTCCTGGCCCTCTGAGTAA  
ATGGATGAAGCGAAGAAGAATGGGGTCCTCTGAGTAACAGGTGGATCTA  
GAAAATCCTATAGGAGTCACCAGGGCACGGTGGAGGAGGGTAAGGTACA  
10 GAACTAACAAATAGCCCGAGAAGGGGAAACAGCAGGAGATGATTCCAGAG  
ACGTAGTGACCCCAAGCTGCAAGGGAAAGCATGAGGGGCCAGCAGGAAG  
GCCGACATGGCAGGTTGTCAGCTTCTAGATCGGAAGGCGGGTCACACTTG  
CTCTTTCTATCCTCAGGGCCACCTCCTCGCACAAGGAGTGTCTCCTACATC  
AACATCATCATGCCTTCAGTGTTTGGTACCATCTGTCTCCTGGGCATTGTG  
15 GGAAACTCCACAGTCATTTTTGCCGTGGTGAAGAAATCCAAGCTGCACTG  
GTGCAGCAACGTCCCTGACATCTTCATCATCAACCTCTCTGTGGTGGATCT  
GCTTTTCCTGCTGGGCATGCCTTTCATGATCCACCAGCTCATGGGTAATGG  
TGTCTGGCACTTTGGGGAAACCATGTGCACCCTCATCACAGCCATGGACG  
CCAACAGTCAGTTCACCAGCACCTACATCCTGACTGCTATGGCCATTGACC  
20 GCTACTTGGCCACCGTCCATCCCATCTCCTCCACCAAGTTCCGGAAGCCCT  
CCATGGCCACCCTGGTGATCTGCCTCCTGTGGGCTCTCTCGTTCATTAGCA  
TCACTCCTGTGTGGCTCTATGCCAGGCTTATCCCCTTCCCAGGGGGTGCTG  
TGGGCTGTGGCATCCGCCTACCAAACCCAGATACTGATCTTTACTGGTTCA  
CTCTGTATCAGTTTTTCCTGGCCTTCGCCCTTCCGTTTGTGGTCATCACTGC  
25 TCGGTACGTGAAAATACTACAGCGCATGACGTCTTCGGTGGCCCCAGCCT  
CTCAACGCAGCATCCGGCTTCGGACAAAGAGGGTGACCCGCACAGCCATT  
GCCATCTGTCTGGTCTTCTTTGTGTGCTGGGCGCCCTACTACGTGCTGCAG  
CTGACCCAGTTGTCCATCAGCCGCCCCGACCCTCACATTTCGTCTACCTGTAC  
AATGCGGCCATCAGCTTGGGCTATGCCAACAGCTGCCTCAATCCCTTTGTG  
30 TACATAGTACTCTGTGAGACCTTTCGAAAACGCTTGGTGCTGTGCGGTGAA  
GCCCCGCGCCCAGGGGCAGCTTCGCACGGTCAGCAATGCTCAGACAGCTG  
ACGAGGAGAGGACAGAAAGCAAAGGCACCATGGTGAGCAAGGGCGAGG  
AGCTGTTACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTA  
AACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCT  
35 ACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCCGTG

CCCTGGCCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAAGTGCTTCAG  
 CCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGC  
 CCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAAC  
 TACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACC  
 5 GCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGG  
 GCACAAGCTGGAGTACAACCTACAACAGCCACAACGTCTATATCATGGCCG  
 ACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACAT  
 CGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCC  
 ATCGGCGACGGCCCCGTGCTGCTGCCCCGACAACCACTACCTGAGCACCCA  
 10 GTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGC  
 TGGAGTTCGTGACCGCCGCGGGATCACTCTCGGCATGGACGAGCTGTAC  
 AAGTAA

**SEQ. ID. NO. 28: Human short form/mouse species chimeric MCH1R-linker-**

**15 EGFP**

Nucleic acid sequence start codon and start and stop codons for mouse MCH1R and EGFP, respectively, are highlighted and a 12 amino acid linker sequence is denoted in lower case:

ATGGACCTGGAAGCCTCGCTGCTGCCCACTGGTCCCAATGCCAGCAACAC  
 20 CTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCTCCTCGCACGG  
 GGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTCGGCACCATCT  
 GCCTCCTGGGCATCATCGGGAACCTCCACGGTCATCTTCGCGGTCTGTAAG  
 AAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTTCATCATCAA  
 CCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTTCATGATCCA  
 25 CCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCATGTGCACCC  
 TCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACCTACATCCTG  
 ACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCCATCTCTTCC  
 ACGAAGTTCGGGAAGCCCTCCATGGCCACCCTGGTGATCTGCCTCCTGTG  
 GGCTCTCTCGTTCATTAGCATCACTCCTGTGTGGCTCTATGCCAGGCTTAT  
 30 CCCCTTCCCAGGGGGTGTGTGGGCTGTGGCATCCGCCTACCAAACCCAG  
 ATACTGATCTTTACTGGTTCACCTCTGTATCAGTTTTCTGGCCTTCGCCCT  
 TCCGTTTGTGGTCATCACTGCTGCGTACGTGAAAATACTACAGCGCATGAC  
 GTCTTCGGTGGCCCCAGCCTCTCAACGCAGCATCCGGCTTCGGACAAAGA  
 GGGTGACCCGCACAGCCATTGCCATCTGTCTGGTCTTCTTTGTGTGCTGGG  
 35 CGCCCTACTACGTGCTGCAGCTGACCCAGTTGTCCATCAGCCGCCCCGACC

CTCACATTCTGTCTACCTGTACAATGCGGCCATCAGCTTGGGCTATGCCAAC  
 AGCTGCCTCAATCCCTTTGTGTACATAGTACTCTGTGAGACCTTTCGAAAA  
 CGCTTGGTGCTGTCGGTGAAGCCCGCGGCCAGGGGCAGCTTCGCACGGT  
 CAGCAATGCTCAGACAGCTGACGAGGAGAGGACAGAAAGCAAAGGCACC  
 5 gtcgacggtaccgccccgggatccatcgccaccATGGTGAGCAAGGGCGAGGAGCTGTT  
 CACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCC  
 ACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAA  
 GCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGC  
 CCACCCTCGTGACCACCCTGACCTACGGCGTGCACTGCTTCAGCCGCTAC  
 10 CCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGG  
 CTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGA  
 CCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAG  
 CTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGC  
 TGGAGTACAACACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAG  
 15 AAGAACGGCATCAAGGTGAACCTCAAGATCCGCCACAACATCGAGGACG  
 GCAGCGTGCACTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGAC  
 GGCCCCGTGCTGCTGCCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT  
 GAGCAAAGACCCCAACGAGAAGCGCGATCATATGGTCCTGCTGGAGTTCCG  
 TGACCGCCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA

20

**SEQ. ID. NO. 29: Human long form/mouse species chimeric MCH1R-linker-EGFP**

Nucleic acid sequence start codon and start and stop codons for mouse MCH1R and EGFP, respectively, are highlighted and a 12 amino acid linker sequence is denoted in lower case:

25

ATGTCAGTGGGAGCCATGAAGAAGGGAGTGGGGAGGGCAGTTGGGCTTG  
 GAGGCGGCAGCGGCTGCCAGGCTACGGAGGAAGACCCCCTTCCCAACTGC  
 GGGGCTTGCGCTCCGGGACAAGGTGGCAGGCGCTGGAGGCTGCCGCAGC  
 CTGCGTGGGTGGAGGGGAGCTCAGCTCGGTTGTGGGAGCAGGCGACCGG  
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TAA

Example 2: Generation of Chimeric and Fusion Proteins

DNA vectors encoding fusion proteins between a MCH-R receptor  
35 (MCH1R) and several different superbright variants of Green Fluorescent Protein

(GFP) were generated. GFP variants were fused either via a 12 amino acid linker: TCGACGGTACCGCGGGCCCCGGGATCCATCGCCACC (SEQ. ID. NO. 30), amino acid sequence: VDGTAGPGSIAT (SEQ. ID. NO. 31) (linker fusions) or directly to the C-terminus of MCH1R (direct fusions).

5

#### Mouse MCH1R-linker-GFP Variant Fusion Constructs

Initially, mouse MCH1R was fused in frame via the linker to Enhanced Green Fluorescent Protein (EGFP). MCH1R was PCR-amplified (95°C for 5 minutes; 95°C for 30 seconds, 60°C for 45 seconds, 68°C for 3.5 minutes, for 15 cycles; 68°C for 7 minutes) from a full-length mouse MCH1R genomic DNA lambda clone utilizing a high fidelity polymerase mix (Expand High Fidelity PCR System from Boehringer Mannheim) and PCR primers [MCH1R (Eco RI) 5': GCGAATTCACCATGGATCTGCAAGCCTCG (SEQ. ID. NO. 32), MCH1R (Sal I) 3': GCGTCGACGGTGCCTTTGCTTTCTGTCC (SEQ. ID. NO. 33)] that generated Eco RI and Sal I enzymatic restriction sites at the N- and C-terminus, respectively. The MCH1R N-terminal PCR primer was also designed to introduce a Kozak consensus sequence for translation which contained an Nco I site (5'-ACCATGG-3'), and the MCH1R C-terminal PCR primer was also designed to eliminate the endogenous stop codon present in the mouse MCH1R gene. The resulting PCR product was phenol/chloroform extracted, restriction digested with Eco RI and Sal I, gel purified, and subcloned in frame into the multicloning site of Clontech's pEGFP-N3 vector between Eco RI and Sal I sites. Several resulting clones for this construct were sequenced to identify a clone with an entirely correct nucleotide sequence. This clone was named mMCH1R-I-EGFP for mouse MCH1R-linker-EGFP.

25 An approximately 760 bp Sal I to Not I fragment of mMCH1R-I-EGFP was excised, gel purified, and subcloned into the multicloning site of pBluescript (SK+) (Stratagene) between Sal I and Not I sites. An approximately 710 bp Nco I to Bsr G1 fragment of EGFP was excised from the resulting pBluescript-EGFP vector and replaced with the corresponding Nco I to Bsr G1 fragment of either Emerald, Topaz, or W1B (other superbright GFP variants), which were excised from vectors pRSET-Emerald, pRSET-Topaz, and pRSET-W1B, respectively. pRSET-Emerald, pRSET-Topaz, and pRSET-W1B were obtained from Aurora Biosciences Co. Sal I to Not I fragments containing either Emerald, Topaz, or W1B were excised from the resulting pBluescript-Emerald, pBluescript-Topaz, and pBluescript-W1B vectors, respectively. Appropriate fragments were gel purified and subcloned into mMCH1R-

35

l-EGFP digested with Sal I and Not I, replacing the Sal I to Not I EGFP fragment with the corresponding Sal I to Not I fragment from either Emerald, Topaz, or W1B. Several clones for each construct were sequenced to confirm the presence of the appropriate GFP variant. The resulting vectors were named mMCH1R-l-Emerald, mMCH1R-l-Topaz, and mMCH1R-l-W1B for mouse MCH1R-linker-Emerald, mouse MCH1R-linker-Topaz, and mouse MCH1R-linker-W1B, respectively.

#### Mouse MCH1R/GFP Variant Direct Fusion Constructs

A two step PCR strategy was employed to generate the direct fusion constructs. First, mouse MCH1R, EGFP, and Emerald were PCR-amplified from a full-length mouse MCH1R genomic DNA lambda clone, Clontech's pEGFP-N3 vector, and Aurora's pRSET-Emerald vector, respectively. Mouse MCH1R was PCR-amplified according to the previously mentioned conditions utilizing the same N-terminal PCR primer [MCH1R (Eco RI) 5': GCGAATTCACCATGGATCTGCA AGCCTCG (SEQ. ID. NO. 32)], but in this case a different C-terminal PCR primer was employed. The C-terminal PCR primer [MCH1R (EGFP/Emerald) 3': CCTTGCTCACCATGGTGCCTTTGCTTTCTGTCC (SEQ. ID. NO. 34)] eliminated the endogenous stop codon of mouse MCH1R as before and introduced a region of nucleotide sequence complementary to the nucleotide sequence of the N-terminus of EGFP.

EGFP and Emerald were PCR-amplified (95°C for 5 minutes; 95°C for 30 seconds, 60°C for 45 seconds, 68°C for 1.5 minutes, for 15 cycles; 68°C for 7 minutes) separately with a high fidelity polymerase mix (Advantage HF-2 from Clontech) from their respective templates utilizing a common N-terminal PCR primer [EGFP/Emerald (MCH1R) 5': CAGAAAGCAAAGGCACCATGGTGAGCAA GGGCGAGGAGC (SEQ. ID. NO. 35)] that generated a region of nucleotide sequence complementary to the C-terminus of mouse MCH1R and C-terminal PCR primers [EGFP 3': GGCGGATCCTCTAGAGTCGCGGCC (SEQ. ID. NO. 36), or Emerald (EGFP) 3': GCTCTAGAGTCGCGGCCGCTTACTTGTACAGCTCGTCC (SEQ. ID. NO. 37)] that generated a Not I site at the C-terminus. The resulting PCR products were electrophoresed on an agarose gel and the appropriate fragments were gel purified.

In a second PCR step, PCR reactions were set up between the previously generated mouse MCH1R and EGFP, or mouse MCH1R and Emerald PCR products. Following an initial 5 minute denaturation step at 95°C, two rounds of



thermocycling (95°C for 30 seconds, 60°C for 45 seconds, 68°C for 4 minutes) were performed in the absence of PCR primers. This allowed the mouse MCH1R and GFP variants to anneal at their complementary regions and to be filled in by the high fidelity polymerase mix (Expand High Fidelity PCR System from Boehringer  
5 Mannheim), yielding double stranded template DNA.

Subsequently, the common N-terminal mouse MCH1R [MCH1R (Eco RI) 5': GCGAATTCACCATGGATCTGCAAGCCTCG (SEQ. ID. NO. 32)] and appropriate C-terminal PCR primers [EGFP 3': GGCGGATCCTCTAGAGTC GCGGCC (SEQ. ID. NO. 36) or Emerald (EGFP) 3': GCTCTAGAGTCGCGG  
10 CCGCTTACTTGTACAGCTCGTCC (SEQ. ID. NO. 37)] were added to the reactions and thermocycling was continued for an additional fifteen cycles followed by a final extension at 68°C for 7 minutes. The resulting PCR products were phenol/chloroform extracted, restriction digested with Eco RI and Not I, electrophoresed on an agarose gel, and appropriate fragments were gel purified.

15 These Eco RI to Not I fragments represent direct fusions between either mouse MCH1R and EGFP, or mouse MCH1R and Emerald. Clontech's pEGFP-N3 vector was restriction digested with Eco RI and Not I liberating an approximately 780 bp Eco RI to Not I EGFP fragment. This restriction digest was electrophoresed on an agarose gel and the approximately 3.9 Kb pEGFP-N3 vector  
20 backbone was gel purified. Eco RI to Not I mouse MCH1R/EGFP or mouse MCH1R/Emerald direct fusion fragments were subcloned into the pEGFP-N3 vector backbone between Eco RI and Not I sites. Several resulting clones for each of these two constructs were sequenced to identify clones with correct nucleotide sequence; however, no clones with entirely correct nucleotide sequences were identified.  
25 Fortunately, several clones for each of the two constructs only had nucleotide mismatches in the intron region of mouse MCH1R, and therefore, were not expected to effect the functionality of the resulting fusion proteins. These clones were named mMCH1R/EGFP and mMCH1R/Emerald for mouse MCH1R/EGFP direct fusion and mouse MCH1R/Emerald direct fusion, respectively.

30

#### Human Short and Long Form/Mouse Species Chimeric

##### MCH1R-linker-GFP Variant Fusion Constructs

The initial mouse MCH1R-linker-GFP variant fusion constructs were modified to generate both human short form and human long form/mouse species

chimeric MCH1R-linker-GFP variant fusion constructs. An approximately 1.7 kb Hind III to Bsp EI fragment of the mouse MCH1R gene containing exon 1, the intron, and 127 amino acids of exon 2 was excised from the various mouse MCH1R-linker-GFP variant fusion constructs and replaced by either an approximately 470 bp Hind  
5 III to Bsp EI fragment from the wild-type human MCH1R short form or an approximately 670 bp Hind III to Bsp EI fragment from the wild-type human MCH1R long form.

Several clones for each construct were sequenced to confirm the presence of the N-terminal region of either the human MCH1R short or long forms.  
10 These clones were named hshort/mMCH1R-l-GFP variant or hlong/mMCH1R-l-GFP variant for human short form/mouse species chimeric MCH1R-linker-GFP variant and human long form/mouse species chimeric MCH1R-linker-GFP variant, respectively.

15 Example 3: Functional Evaluation of MCH1R/GFP Variant Fusion Proteins

Both HEK293 Aequorin (National Institutes of Health) and CHO mammalian cell lines were transiently transfected with the various MCH1R/GFP variant fusion constructs, as well as the appropriate control constructs. Transfection was performed using Lipofectamine 2000 (Gibco BRL) per the manufacturer  
20 recommended protocol. Approximately 48 hours after transfection cells were harvested, stimulated with various concentrations of human MCH, and assayed for either aequorin bioluminescence (HEK293 Aequorin cells) or cAMP production (CHO cells). Aequorin bioluminescence is a representative measure of intracellular  $\text{Ca}^{2+}$  mobilization. cAMP production was measured with the Adenylyl Cyclase  
25 Activation FlashPlate Assay (NEN Life Science Products, Inc.).

Following transient transfection of the mMCH1R-linker-EGFP construct (MCH-R-l-EGFP) into HEK293 Aequorin cells, the resulting fusion protein exhibited functional activity comparable to that of the wild-type human MCH1R short form (MCH-R wt). By this functional assay, the  $\text{EC}_{50}$  value for mMCH1R-l-EGFP  
30 was nearly identical to that of the wild-type human short form receptor (Figure 1).

Following transient transfections of the mMCH1R-l-EGFP and mMCH1R/EGFP fusion constructs into CHO cells, the resulting fusion proteins exhibited functional activity comparable to that of the wild-type human MCH1R short form. By this functional assay, the  $\text{EC}_{50}$  values for mMCH1R-l-EGFP and  
35 mMCH1R/EGFP were comparable to that of the wild-type human receptor (Table 1).

Transient transfections with the corresponding Emerald constructs yielded similar results (data not shown).

Table 1

5

Receptor	EC <sub>50</sub> (nM)
Wild-type Human MCH1R Short Form	2.166
Mouse MCH1R/EGFP	0.819
Mouse MCH1R-l-EGFP	3.199

Following transient transfections of the human short form/mouse species chimeric MCH1R-l-EGFP (HuShort/mMCH1R-l-EGFP) and human long form/mouse species chimeric MCH1R-l-EGFP (HuLong/mMCH1R-l-EGFP) constructs into HEK293 cells, the resulting fusion proteins exhibited functional activity comparable to that of the wild-type human MCH1R short and long forms, respectively. By this functional assay, the EC<sub>50</sub> value for each fusion proteins was nearly identical to that of the corresponding wild-type human receptor (Table 2).

15

Table 2

Receptor	EC <sub>50</sub> (nM)
Wild-type Human MCH1R Short Form	22.27
HuShort/mMCH1R-l-EGFP	19.54
Wild-type Human MCH1R Long Form	196.7
HuLong/mMCH1R-l-EGFP Form	217.5

Following transient transfections of the human short form/mouse species chimeric MCH1R-l-EGFP (HuShort/mMCH1R-l-EGFP) and human long form/mouse species chimeric MCH1R-l-EGFP (HuLong/mMCH1R-l-EGFP) constructs into CHO cells, the resulting fusion proteins exhibited functional activity comparable to or less than that of the wild-type human MCH1R short and long forms, respectively (Table 3). By this functional assay, the EC<sub>50</sub> value for the human short form/mouse species chimeric MCH1R-l-EGFP fusion protein was comparable to that of the corresponding wild-type human receptor, whereas, the human long form/mouse

species chimeric MCH1R-l-EGFP fusion protein had an EC<sub>50</sub> value approximately 7.5-fold higher than that of its corresponding wild-type control.

Table 3

5

Receptor	EC <sub>50</sub> (nM)
Wild-type Human MCH1R Short Form	1.029
Wild-type Human MCH1R Long Form	1.515
HuShort/mMCH1R-l-EGFP	1.565
HuLong/mMCH1R-l-EGFP	11.580

Transient expression of all the MCH1R/GFP variant fusion proteins that underwent functional evaluation resulted in fluorescence primarily associated with the plasma membrane in both HEK293 and CHO cells (data not shown). This pattern of fluorescence is consistent with a predominant membrane associated localization.

10

#### Example 4: Generation of Stable Cell Lines

Wild-type CHO cells were transfected using SuperFect (Qiagen) and either mouse MCH-1R-EGFP or human short/mouse species chimeric MCH-1R-EGFP. Forty-eight hours after transfection, transfected cells were subjected to positive selection for approximately ten days in media containing G418. Following selection, MCH-1R-EGFP expressing CHO cells were bulk sorted by Fluorescence Assisted Cell Sorting (FACS) for one or two rounds on the basis of fluorescence intensity to increase the population of cells expressing EGFP. Following bulk sorts, individual clones of varying fluorescence intensities were isolated by FACS and expanded.

20

Fluorometric Microvolume Assay Technology (FMAT) was initially employed to screen a large number of stable clones by whole cell binding with a fluorescently labeled MCH derivative (SymJz-MCH, PE Biosystems) to identify those clones with good specific binding windows. Several clones exhibiting specific binding windows greater than 3-fold were further evaluated for MCH binding with the SPA-based Binding Assay. Cells from individual clones were dissociated in enzyme free dissociation media and cell membranes were prepared and subsequently tested for

25

their ability to bind [ $^{125}$ I]Phe<sup>13</sup>Tyr<sup>19</sup>-MCH in the presence of human MCH. CHO cell lines expressing either mouse MCH-1R-EGFP or human short/mouse species chimeric MCH-1R-EGFP (Figure 4) displayed IC50 values with MCH that were indistinguishable from the corresponding IC50 values obtained with a CHO cell line  
5 expressing the wild-type human short isoform of MCH-1R.

The functional activity of these clones was evaluated with the cAMP Flashplate Assay (Figures 2 and 3). CHO cell lines expressing either mouse MCH-1R-EGFP (Figure 2) or human short/mouse species chimeric MCH-1R-EGFP (Figure 3) displayed EC50 values with human MCH that were indistinguishable from the  
10 EC50 value obtained with a CHO cell line expressing the wild-type human short isoform of MCH-1R.

The subcellular localization of the MCH-1R-EGFP fusion proteins were determined by confocal microscopy utilizing EGFP fluorescence as a marker for MCH-1R expression. CHO cell lines stably expressing either mouse MCH-1R-EGFP  
15 or human short/mouse species chimeric MCH-1R-EGFP displayed EGFP fluorescence primarily associated with the plasma membrane, demonstrating that these MCH-1R-EGFP fusion proteins are primarily associated with the plasma membrane.

Other embodiments are within the following claims. While several  
20 embodiments have been shown and described, various modifications may be made without departing from the spirit and scope of the present invention.

## WHAT IS CLAIMED IS:

1. A fusion protein comprising:
  - a) a melanin concentrating hormone receptor polypeptide  
5 region comprising a sequence selected from the group consisting of: SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3, SEQ. ID. NO. 4, and SEQ. ID. NO. 5; and
  - b) a fluorescent polypeptide region joined directly, or  
10 though a linker, to the carboxy side of said melanin concentrating hormone receptor polypeptide region.
2. The protein of claim 1, wherein said fluorescent polypeptide  
region consists of an amino acid sequence selected from the group consisting of SEQ.  
ID. NO. 6, SEQ. ID. NO. 7, SEQ. ID. NO. 8, SEQ. ID. NO. 9, and SEQ. ID. NO. 10.
- 15 3. The protein of claim 2, wherein said melanin concentrating  
hormone polypeptide region consists of a sequence selected from the group consisting  
of: SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3, SEQ. ID. NO. 4, and SEQ. ID.  
NO. 5.
- 20 4. The protein of claim 3, wherein said protein consists essentially  
of said melanin concentrating hormone receptor polypeptide region and said  
fluorescent polypeptide region.
- 25 5. The protein of claim 4, wherein said protein consists of the  
amino acid sequence of SEQ. ID. NO. 11 or SEQ. ID. NO. 12.
6. The protein of claim 1, wherein said melanin concentrating  
hormone polypeptide region is a chimeric polypeptide comprising (a) an MCH  
binding region from a first species and (b) a transmembrane and intracellular domain  
30 region from a second species joined directly, or though a linker, to the carboxy side of  
said MCH binding region.
7. The protein of claim 6, wherein said fluorescent polypeptide  
region consists of an amino acid sequence selected from the group consisting of: SEQ.  
35 ID. NO. 6, SEQ. ID. NO. 7, SEQ. ID. NO. 8, SEQ. ID. NO. 9, and SEQ. ID. NO. 10.

8. The protein of claim 7, wherein said protein consists of the amino acid sequence of SEQ. ID. NO. 13 or SEQ. ID. NO. 14.

5                   9. A chimeric melanin concentrating hormone protein comprising:  
a) a melanin concentrating hormone binding region  
characteristic of a human melanin concentrating hormone receptor;  
b) a transmembrane domain characteristic of a non-human  
melanin concentrating hormone receptor; and  
10                   c) an intracellular domain characteristic of a non-human  
melanin concentrating hormone receptor.

10. The protein of claim 9, wherein said protein comprises a melanin concentrating hormone receptor polypeptide having a sequence similarity of  
15 at least 75% with either SEQ. ID. NO. 4 or SEQ. ID. NO. 5.

11. The protein of claim 10, wherein said protein comprises the sequence of SEQ. ID. NO. 4 or SEQ. ID. NO. 5.

20                   12. The protein of claim 11, wherein said protein consists of the sequence of SEQ. ID. NO. 4 or SEQ. ID. NO. 5.

13. A nucleic acid comprising a nucleotide sequence encoding for the protein of claim 1.  
25

14. The nucleic acid of claim 13, wherein said nucleotide sequence is a contiguous sequence.

15. The nucleic acid of claim 13, wherein said nucleotide sequence  
30 is selected from the group consisting of SEQ. ID. NO. 26, SEQ. ID. NO. 27, SEQ. ID. NO. 28 and SEQ. ID. NO. 29.

16. A nucleic acid comprising a nucleotide sequence encoding for the protein of claim 9.  
35

17. The nucleic acid of claim 16, wherein said nucleotide sequence is a contiguous sequence.
18. The nucleic acid of claim 16, wherein said nucleotide sequence is selected from the group consisting of SEQ. ID. NO. 19 and SEQ. ID. NO. 20.
19. An expression vector comprising the nucleic acid of claim 13.
20. An expression vector comprising the nucleic acid of claim 16.
21. A recombinant cell comprising the nucleic acid of claim 13.
22. The recombinant cell of claim 21, wherein said nucleic acid is present in an expression vector.
23. The recombinant cell of claim 21, wherein said nucleic acid is present in the genome of said cell.
24. A recombinant cell comprising the nucleic acid of claim 16.
25. The recombinant cell of claim 24, wherein said nucleic acid is present in an expression vector.
26. The recombinant cell of claim 24, wherein said nucleic acid is present in the genome of said cell.
27. A non-human transgenic animal comprising the nucleic acid of claim 13.
28. A non-human transgenic animal comprising the nucleic acid of claim 16.
29. A method for assaying for melanin concentrating hormone receptor active compounds comprising the steps of:



a) contacting the cell of claim 21 with a test preparation comprising one or more test compounds; and

b) measuring the effect of said test preparation on one or more melanin concentrating hormone receptor activities.

5

30. A method for assaying for melanin concentrating hormone receptor active compounds comprising the steps of:

a) contacting the cell of claim 24 with a test preparation comprising one or more test compounds; and

10 b) measuring the effect of said test preparation on one or more melanin concentrating hormone receptor activities.

mMCH1R-I-EGFP Aequorin Assay

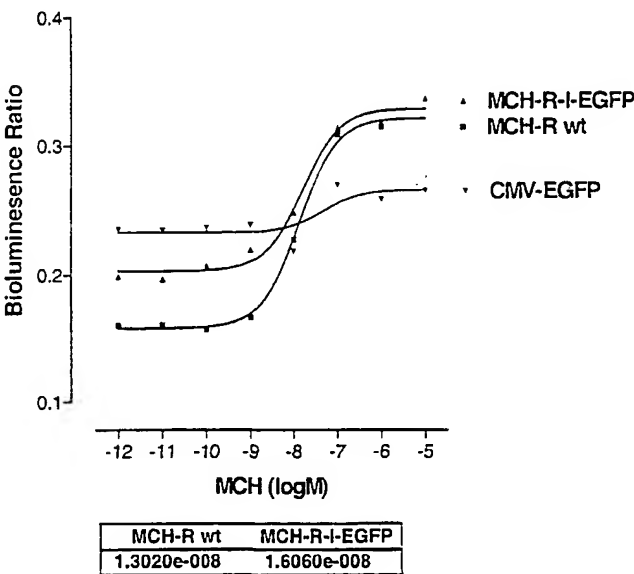


Fig. 1

2/3

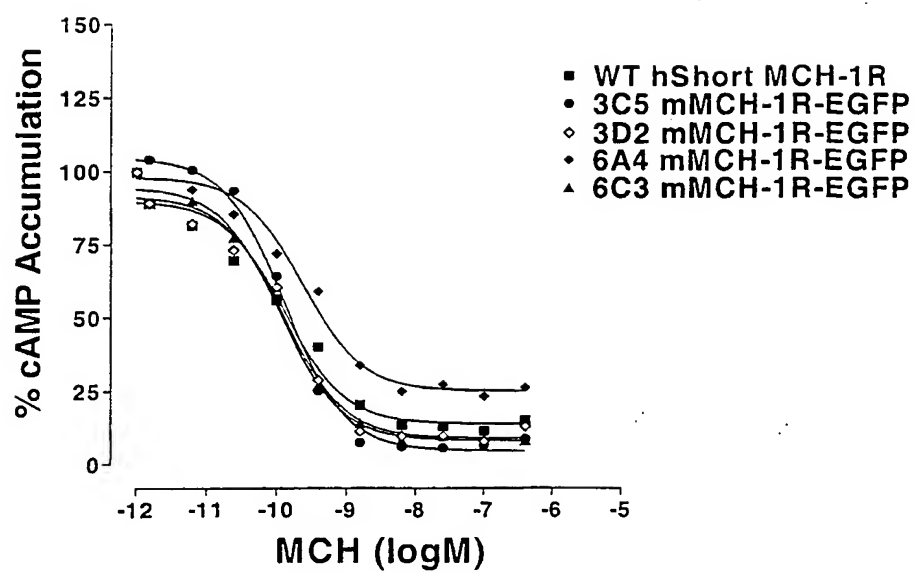


Fig. 2

3/3

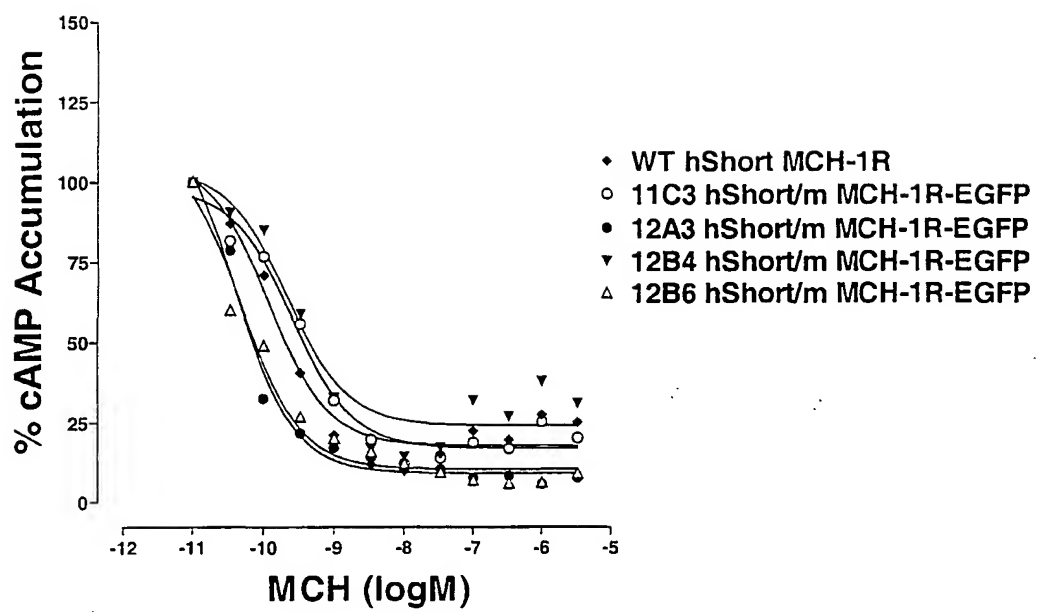


Fig. 3

## SEQUENCE LISTING

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<120> MELANIN CONCENTRATING HORMONE RECEPTOR  
CHIMERIC AND FUSION PROTEINS

&lt;130&gt; 20652 PCT

&lt;150&gt; 60/189,698

&lt;151&gt; 2000-03-15

&lt;160&gt; 37

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 Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser Arg Pro Thr Leu Thr  
 275 280 285  
 Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu Gly Tyr Ala Asn Ser  
 290 295 300

Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys Glu Thr Phe Arg Lys  
 305 310 315 320  
 Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln Gly Gln Leu Arg Ala  
 325 330 335  
 Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg Thr Glu Ser Lys Gly  
 340 345 350  
 Thr

<210> 3  
 <211> 353  
 <212> PRT  
 <213> Mouse

<400> 3  
 Met Asp Leu Gln Ala Ser Leu Leu Ser Thr Gly Pro Asn Ala Ser Asn  
 1 5 10 15  
 Ile Ser Asp Gly Gln Asp Asn Phe Thr Leu Ala Gly Pro Pro Pro Arg  
 20 25 30  
 Thr Arg Ser Val Ser Tyr Ile Asn Ile Ile Met Pro Ser Val Phe Gly  
 35 40 45  
 Thr Ile Cys Leu Leu Gly Ile Val Gly Asn Ser Thr Val Ile Phe Ala  
 50 55 60  
 Val Val Lys Lys Ser Lys Leu His Trp Cys Ser Asn Val Pro Asp Ile  
 65 70 75 80  
 Phe Ile Ile Asn Leu Ser Val Val Asp Leu Leu Phe Leu Leu Gly Met  
 85 90 95  
 Pro Phe Met Ile His Gln Leu Met Gly Asn Gly Val Trp His Phe Gly  
 100 105 110  
 Glu Thr Met Cys Thr Leu Ile Thr Ala Met Asp Ala Asn Ser Gln Phe  
 115 120 125  
 Thr Ser Thr Tyr Ile Leu Thr Ala Met Ala Ile Asp Arg Tyr Leu Ala  
 130 135 140  
 Thr Val His Pro Ile Ser Ser Thr Lys Phe Arg Lys Pro Ser Met Ala  
 145 150 155 160  
 Thr Leu Val Ile Cys Leu Leu Trp Ala Leu Ser Phe Ile Ser Ile Thr  
 165 170 175  
 Pro Val Trp Leu Tyr Ala Arg Leu Ile Pro Phe Pro Gly Gly Ala Val  
 180 185 190  
 Gly Cys Gly Ile Arg Leu Pro Asn Pro Asp Thr Asp Leu Tyr Trp Phe  
 195 200 205  
 Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu Pro Phe Val Val Ile  
 210 215 220  
 Thr Ala Ala Tyr Val Lys Ile Leu Gln Arg Met Thr Ser Ser Val Ala  
 225 230 235 240  
 Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr Lys Arg Val Thr Arg  
 245 250 255  
 Thr Ala Ile Ala Ile Cys Leu Val Phe Phe Val Cys Trp Ala Pro Tyr  
 260 265 270  
 Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser Arg Pro Thr Leu Thr  
 275 280 285  
 Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu Gly Tyr Ala Asn Ser  
 290 295 300  
 Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys Glu Thr Phe Arg Lys  
 305 310 315 320  
 Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln Gly Gln Leu Arg Thr  
 325 330 335  
 Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg Thr Glu Ser Lys Gly  
 340 345 350  
 Thr

<210> 4  
 <211> 353  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Human short form/mouse species chimeric MCH1R

<400> 4  
 Met Asp Leu Glu Ala Ser Leu Leu Pro Thr Gly Pro Asn Ala Ser Asn  
 1 5 10 15  
 Thr Ser Asp Gly Pro Asp Asn Leu Thr Ser Ala Gly Ser Pro Pro Arg  
 20 25 30  
 Thr Gly Ser Ile Ser Tyr Ile Asn Ile Ile Met Pro Ser Val Phe Gly  
 35 40 45  
 Thr Ile Cys Leu Leu Gly Ile Ile Gly Asn Ser Thr Val Ile Phe Ala  
 50 55 60  
 Val Val Lys Lys Ser Lys Leu His Trp Cys Asn Asn Val Pro Asp Ile  
 65 70 75 80  
 Phe Ile Ile Asn Leu Ser Val Val Asp Leu Leu Phe Leu Leu Gly Met  
 85 90 95  
 Pro Phe Met Ile His Gln Leu Met Gly Asn Gly Val Trp His Phe Gly  
 100 105 110  
 Glu Thr Met Cys Thr Leu Ile Thr Ala Met Asp Ala Asn Ser Gln Phe  
 115 120 125  
 Thr Ser Thr Tyr Ile Leu Thr Ala Met Ala Ile Asp Arg Tyr Leu Ala  
 130 135 140  
 Thr Val His Pro Ile Ser Ser Thr Lys Phe Arg Lys Pro Ser Met Ala  
 145 150 155 160  
 Thr Leu Val Ile Cys Leu Leu Trp Ala Leu Ser Phe Ile Ser Ile Thr  
 165 170 175  
 Pro Val Trp Leu Tyr Ala Arg Leu Ile Pro Phe Pro Gly Gly Ala Val  
 180 185 190  
 Gly Cys Gly Ile Arg Leu Pro Asn Pro Asp Thr Asp Leu Tyr Trp Phe  
 195 200 205  
 Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu Pro Phe Val Val Ile  
 210 215 220  
 Thr Ala Ala Tyr Val Lys Ile Leu Gln Arg Met Thr Ser Ser Val Ala  
 225 230 235 240  
 Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr Lys Arg Val Thr Arg  
 245 250 255  
 Thr Ala Ile Ala Ile Cys Leu Val Phe Phe Val Cys Trp Ala Pro Tyr  
 260 265 270  
 Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser Arg Pro Thr Leu Thr  
 275 280 285  
 Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu Gly Tyr Ala Asn Ser  
 290 295 300  
 Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys Glu Thr Phe Arg Lys  
 305 310 315 320  
 Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln Gly Gln Leu Arg Thr  
 325 330 335  
 Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg Thr Glu Ser Lys Gly  
 340 345 350  
 Thr

<210> 5  
 <211> 422  
 <212> PRT  
 <213> Artificial Sequence

<220>



<223> Human long form/mouse species chimeric MCH1R

<400> 5

```

Met Ser Val Gly Ala Met Lys Lys Gly Val Gly Arg Ala Val Gly Leu
 1      5      10      15
Gly Gly Gly Ser Gly Cys Gln Ala Thr Glu Glu Asp Pro Leu Pro Asn
 20      25      30
Cys Gly Ala Cys Ala Pro Gly Gln Gly Gly Arg Arg Trp Arg Leu Pro
 35      40      45
Gln Pro Ala Trp Val Glu Gly Ser Ser Ala Arg Leu Trp Glu Gln Ala
 50      55      60
Thr Gly Thr Gly Trp Met Asp Leu Glu Ala Ser Leu Leu Pro Thr Gly
 65      70      75      80
Pro Asn Ala Ser Asn Thr Ser Asp Gly Pro Asp Asn Leu Thr Ser Ala
 85      90      95
Gly Ser Pro Pro Arg Thr Gly Ser Ile Ser Tyr Ile Asn Ile Ile Met
100      105      110
Pro Ser Val Phe Gly Thr Ile Cys Leu Leu Gly Ile Ile Gly Asn Ser
115      120      125
Thr Val Ile Phe Ala Val Val Lys Lys Ser Lys Leu His Trp Cys Asn
130      135      140
Asn Val Pro Asp Ile Phe Ile Ile Asn Leu Ser Val Val Asp Leu Leu
145      150      155      160
Phe Leu Leu Gly Met Pro Phe Met Ile His Gln Leu Met Gly Asn Gly
165      170      175
Val Trp His Phe Gly Glu Thr Met Cys Thr Leu Ile Thr Ala Met Asp
180      185      190
Ala Asn Ser Gln Phe Thr Ser Thr Tyr Ile Leu Thr Ala Met Ala Ile
195      200      205
Asp Arg Tyr Leu Ala Thr Val His Pro Ile Ser Ser Thr Lys Phe Arg
210      215      220
Lys Pro Ser Met Ala Thr Leu Val Ile Cys Leu Leu Trp Ala Leu Ser
225      230      235      240
Phe Ile Ser Ile Thr Pro Val Trp Leu Tyr Ala Arg Leu Ile Pro Phe
245      250      255
Pro Gly Gly Ala Val Gly Cys Gly Ile Arg Leu Pro Asn Pro Asp Thr
260      265      270
Asp Leu Tyr Trp Phe Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu
275      280      285
Pro Phe Val Val Ile Thr Ala Ala Tyr Val Lys Ile Leu Gln Arg Met
290      295      300
Thr Ser Ser Val Ala Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr
305      310      315      320
Lys Arg Val Thr Arg Thr Ala Ile Ala Ile Cys Leu Val Phe Phe Val
325      330      335
Cys Trp Ala Pro Tyr Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser
340      345      350
Arg Pro Thr Leu Thr Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu
355      360      365
Gly Tyr Ala Asn Ser Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys
370      375      380
Glu Thr Phe Arg Lys Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln
385      390      395      400
Gly Gln Leu Arg Thr Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg
405      410      415
Thr Glu Ser Lys Gly Thr
420

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<210> 6

<211> 238

<212> PRT

<213> Aequorea Victoria

```

<400> 6
Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
 1      5      10      15
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
 20      25      30
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
 35      40      45
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
 50      55      60
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
 65      70      75      80
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
 85      90      95
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
 100     105     110
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
 115     120     125
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
 130     135     140
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
 145     150     155     160
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
 165     170     175
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
 180     185     190
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
 195     200     205
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
 210     215     220
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
 225     230     235

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<210> 7
<211> 239
<212> PRT
<213> Artificial Sequence

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<220>
<223> GFP derivative

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```

<400> 7
Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1      5      10      15
Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
 20      25      30
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
 35      40      45
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
 50      55      60
Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
 65      70      75      80
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
 85      90      95
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
 100     105     110
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
 115     120     125
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
 130     135     140
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
 145     150     155     160

```

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser  
 165 170 175  
 Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly  
 180 185 190  
 Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu  
 195 200 205  
 Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe  
 210 215 220  
 Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys  
 225 230 235

<210> 8  
 <211> 239  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> GFP derivative

<400> 8  
 Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu  
 1 5 10 15  
 Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly  
 20 25 30  
 Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile  
 35 40 45  
 Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr  
 50 55 60  
 Leu Thr Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys  
 65 70 75 80  
 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu  
 85 90 95  
 Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu  
 100 105 110  
 Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly  
 115 120 125  
 Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr  
 130 135 140  
 Asn Tyr Asn Ser His Lys Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn  
 145 150 155 160  
 Gly Ile Lys Val Asn Phe Lys Thr Arg His Asn Ile Glu Asp Gly Ser  
 165 170 175  
 Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly  
 180 185 190  
 Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu  
 195 200 205  
 Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe  
 210 215 220  
 Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys  
 225 230 235

<210> 9  
 <211> 239  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> GFP derivative

<400> 9  
 Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu  
 1 5 10 15

```

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
      20      25      30
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
      35      40      45
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
      50      55      60
Phe Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Arg
      65      70      75      80
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
      85      90      95
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
      100      105      110
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
      115      120      125
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
      130      135      140
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
      145      150      155      160
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
      165      170      175
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
      180      185      190
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu
      195      200      205
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
      210      215      220
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
      225      230      235

```

&lt;210&gt; 10

&lt;211&gt; 239

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; GFP derivative

&lt;400&gt; 10

```

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
      1      5      10      15
Val Glu Leu Asp Gly Asp Val Asn Gly His Arg Phe Ser Val Ser Gly
      20      25      30
Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
      35      40      45
Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
      50      55      60
Leu Thr Trp Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
      65      70      75      80
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
      85      90      95
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
      100      105      110
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
      115      120      125
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
      130      135      140
Asn Tyr Ile Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn
      145      150      155      160
Gly Ile Lys Ala His Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
      165      170      175
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
      180      185      190

```

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu  
 195 200 205  
 Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe  
 210 215 220  
 Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys  
 225 230 235

<210> 11  
 <211> 604  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Mouse MCH1R-linker-EGFP

<400> 11  
 Met Asp Leu Gln Ala Ser Leu Leu Ser Thr Gly Pro Asn Ala Ser Asn  
 1 5 10 15  
 Ile Ser Asp Gly Gln Asp Asn Phe Thr Leu Ala Gly Pro Pro Pro Arg  
 20 25 30  
 Thr Arg Ser Val Ser Tyr Ile Asn Ile Ile Met Pro Ser Val Phe Gly  
 35 40 45  
 Thr Ile Cys Leu Leu Gly Ile Val Gly Asn Ser Thr Val Ile Phe Ala  
 50 55 60  
 Val Val Lys Lys Ser Lys Leu His Trp Cys Ser Asn Val Pro Asp Ile  
 65 70 75 80  
 Phe Ile Ile Asn Leu Ser Val Val Asp Leu Leu Phe Leu Leu Gly Met  
 85 90 95  
 Pro Phe Met Ile His Gln Leu Met Gly Asn Gly Val Trp His Phe Gly  
 100 105 110  
 Glu Thr Met Cys Thr Leu Ile Thr Ala Met Asp Ala Asn Ser Gln Phe  
 115 120 125  
 Thr Ser Thr Tyr Ile Leu Thr Ala Met Ala Ile Asp Arg Tyr Leu Ala  
 130 135 140  
 Thr Val His Pro Ile Ser Ser Thr Lys Phe Arg Lys Pro Ser Met Ala  
 145 150 155 160  
 Thr Leu Val Ile Cys Leu Leu Trp Ala Leu Ser Phe Ile Ser Ile Thr  
 165 170 175  
 Pro Val Trp Leu Tyr Ala Arg Leu Ile Pro Phe Pro Gly Gly Ala Val  
 180 185 190  
 Gly Cys Gly Ile Arg Leu Pro Asn Pro Asp Thr Asp Leu Tyr Trp Phe  
 195 200 205  
 Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu Pro Phe Val Val Ile  
 210 215 220  
 Thr Ala Ala Tyr Val Lys Ile Leu Gln Arg Met Thr Ser Ser Val Ala  
 225 230 235 240  
 Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr Lys Arg Val Thr Arg  
 245 250 255  
 Thr Ala Ile Ala Ile Cys Leu Val Phe Phe Val Cys Trp Ala Pro Tyr  
 260 265 270  
 Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser Arg Pro Thr Leu Thr  
 275 280 285  
 Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu Gly Tyr Ala Asn Ser  
 290 295 300  
 Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys Glu Thr Phe Arg Lys  
 305 310 315 320  
 Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln Gly Gln Leu Arg Thr  
 325 330 335  
 Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg Thr Glu Ser Lys Gly  
 340 345 350  
 Thr Val Asp Gly Thr Ala Gly Pro Gly Ser Ile Ala Thr Met Val Ser  
 355 360 365

Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu  
 370 375 380  
 Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu  
 385 390 395 400  
 Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr  
 405 410 415  
 Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr  
 420 425 430  
 Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp  
 435 440 445  
 Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile  
 450 455 460  
 Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe  
 465 470 475 480  
 Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe  
 485 490 495  
 Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn  
 500 505 510  
 Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys  
 515 520 525  
 Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu  
 530 535 540  
 Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu  
 545 550 555 560  
 Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp  
 565 570 575  
 Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala  
 580 585 590  
 Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys  
 595 600

<210> 12  
 <211> 592  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Mouse MCH1R/EGFP

<400> 12  
 Met Asp Leu Gln Ala Ser Leu Leu Ser Thr Gly Pro Asn Ala Ser Asn  
 1 5 10 15  
 Ile Ser Asp Gly Gln Asp Asn Phe Thr Leu Ala Gly Pro Pro Pro Arg  
 20 25 30  
 Thr Arg Ser Val Ser Tyr Ile Asn Ile Ile Met Pro Ser Val Phe Gly  
 35 40 45  
 Thr Ile Cys Leu Leu Gly Ile Val Gly Asn Ser Thr Val Ile Phe Ala  
 50 55 60  
 Val Val Lys Lys Ser Lys Leu His Trp Cys Ser Asn Val Pro Asp Ile  
 65 70 75 80  
 Phe Ile Ile Asn Leu Ser Val Val Asp Leu Leu Phe Leu Leu Gly Met  
 85 90 95  
 Pro Phe Met Ile His Gln Leu Met Gly Asn Gly Val Trp His Phe Gly  
 100 105 110  
 Glu Thr Met Cys Thr Leu Ile Thr Ala Met Asp Ala Asn Ser Gln Phe  
 115 120 125  
 Thr Ser Thr Tyr Ile Leu Thr Ala Met Ala Ile Asp Arg Tyr Leu Ala  
 130 135 140  
 Thr Val His Pro Ile Ser Ser Thr Lys Phe Arg Lys Pro Ser Met Ala  
 145 150 155 160  
 Thr Leu Val Ile Cys Leu Leu Trp Ala Leu Ser Phe Ile Ser Ile Thr  
 165 170 175

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Pro Val Trp Leu Tyr Ala Arg Leu Ile Pro Phe Pro Gly Gly Ala Val
      180      185      190
Gly Cys Gly Ile Arg Leu Pro Asn Pro Asp Thr Asp Leu Tyr Trp Phe
      195      200      205
Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu Pro Phe Val Val Ile
      210      215      220
Thr Ala Ala Tyr Val Lys Ile Leu Gln Arg Met Thr Ser Ser Val Ala
      225      230      235
Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr Lys Arg Val Thr Arg
      245      250      255
Thr Ala Ile Ala Ile Cys Leu Val Phe Phe Val Cys Trp Ala Pro Tyr
      260      265      270
Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser Arg Pro Thr Leu Thr
      275      280      285
Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu Gly Tyr Ala Asn Ser
      290      295      300
Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys Glu Thr Phe Arg Lys
      305      310      315
Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln Gly Gln Leu Arg Thr
      325      330      335
Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg Thr Glu Ser Lys Gly
      340      345      350
Thr Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile
      355      360      365
Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser
      370      375      380
Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe
      385      390      395
Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
      405      410      415
Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met
      420      425      430
Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
      435      440      445
Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala
      450      455      460
Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
      465      470      475
Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu
      485      490      495
Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys
      500      505      510
Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly
      515      520      525
Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp
      530      535      540
Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala
      545      550      555
Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu
      565      570      575
Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
      580      585      590

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<210> 13
<211> 604
<212> PRT
<213> Artificial Sequence

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<220>
<223> MCH1R-linker-EGFP

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```

<400> 13

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Met	Asp	Leu	Glu	Ala	Ser	Leu	Leu	Pro	Thr	Gly	Pro	Asn	Ala	Ser	Asn
1				5					10					15	
Thr	Ser	Asp	Gly	Pro	Asp	Asn	Leu	Thr	Ser	Ala	Gly	Ser	Pro	Pro	Arg
			20					25					30		
Thr	Gly	Ser	Ile	Ser	Tyr	Ile	Asn	Ile	Ile	Met	Pro	Ser	Val	Phe	Gly
		35					40					45			
Thr	Ile	Cys	Leu	Leu	Gly	Ile	Ile	Gly	Asn	Ser	Thr	Val	Ile	Phe	Ala
	50					55					60				
Val	Val	Lys	Lys	Ser	Lys	Leu	His	Trp	Cys	Asn	Asn	Val	Pro	Asp	Ile
65					70					75					80
Phe	Ile	Ile	Asn	Leu	Ser	Val	Val	Asp	Leu	Leu	Phe	Leu	Leu	Gly	Met
				85					90					95	
Pro	Phe	Met	Ile	His	Gln	Leu	Met	Gly	Asn	Gly	Val	Trp	His	Phe	Gly
			100					105					110		
Glu	Thr	Met	Cys	Thr	Leu	Ile	Thr	Ala	Met	Asp	Ala	Asn	Ser	Gln	Phe
	115						120					125			
Thr	Ser	Thr	Tyr	Ile	Leu	Thr	Ala	Met	Ala	Ile	Asp	Arg	Tyr	Leu	Ala
	130					135						140			
Thr	Val	His	Pro	Ile	Ser	Ser	Thr	Lys	Phe	Arg	Lys	Pro	Ser	Met	Ala
145					150					155					160
Thr	Leu	Val	Ile	Cys	Leu	Leu	Trp	Ala	Leu	Ser	Phe	Ile	Ser	Ile	Thr
				165						170				175	
Pro	Val	Trp	Leu	Tyr	Ala	Arg	Leu	Ile	Pro	Phe	Pro	Gly	Gly	Ala	Val
			180					185					190		
Gly	Cys	Gly	Ile	Arg	Leu	Pro	Asn	Pro	Asp	Thr	Asp	Leu	Tyr	Trp	Phe
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Thr	Leu	Tyr	Gln	Phe	Phe	Leu	Ala	Phe	Ala	Leu	Pro	Phe	Val	Val	Ile
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Thr	Ala	Ala	Tyr	Val	Lys	Ile	Leu	Gln	Arg	Met	Thr	Ser	Ser	Val	Ala
225					230					235					240
Pro	Ala	Ser	Gln	Arg	Ser	Ile	Arg	Leu	Arg	Thr	Lys	Arg	Val	Thr	Arg
			245						250					255	
Thr	Ala	Ile	Ala	Ile	Cys	Leu	Val	Phe	Phe	Val	Cys	Trp	Ala	Pro	Tyr
			260					265					270		
Tyr	Val	Leu	Gln	Leu	Thr	Gln	Leu	Ser	Ile	Ser	Arg	Pro	Thr	Leu	Thr
	275						280					285			
Phe	Val	Tyr	Leu	Tyr	Asn	Ala	Ala	Ile	Ser	Leu	Gly	Tyr	Ala	Asn	Ser
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Cys	Leu	Asn	Pro	Phe	Val	Tyr	Ile	Val	Leu	Cys	Glu	Thr	Phe	Arg	Lys
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Arg	Leu	Val	Leu	Ser	Val	Lys	Pro	Ala	Ala	Gln	Gly	Gln	Leu	Arg	Thr
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Val	Ser	Asn	Ala	Gln	Thr	Ala	Asp	Glu	Glu	Arg	Thr	Glu	Ser	Lys	Gly
			340					345					350		
Thr	Val	Asp	Gly	Thr	Ala	Gly	Pro	Gly	Ser	Ile	Ala	Thr	Met	Val	Ser
	355						360					365			
Lys	Gly	Glu	Glu	Leu	Phe	Thr	Gly	Val	Val	Pro	Ile	Leu	Val	Glu	Leu
	370					375					380				
Asp	Gly	Asp	Val	Asn	Gly	His	Lys	Phe	Ser	Val	Ser	Gly	Glu	Gly	Glu
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Gly	Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Phe	Ile	Cys	Thr	Thr
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Gly	Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr	Leu	Thr	Tyr
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Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro	Asp	His	Met	Lys	Gln	His	Asp
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Phe	Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	Glu	Val	Lys	Phe
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Glu	Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys	Gly	Ile	Asp	Phe
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Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn  
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 Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys  
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 Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu  
                   530                  535                  540  
 Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu  
                   545                  550                  555  
 Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp  
                   565                  570                  575  
 Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala  
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 <223> MCH1R-linker-EGFP

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 Cys Gly Ala Cys Ala Pro Gly Gln Gly Gly Arg Arg Trp Arg Leu Pro  
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 Gln Pro Ala Trp Val Glu Gly Ser Ser Ala Arg Leu Trp Glu Gln Ala  
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 Thr Gly Thr Gly Trp Met Asp Leu Glu Ala Ser Leu Leu Pro Thr Gly  
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 Pro Asn Ala Ser Asn Thr Ser Asp Gly Pro Asp Asn Leu Thr Ser Ala  
                   85                  90                  95  
 Gly Ser Pro Pro Arg Thr Gly Ser Ile Ser Tyr Ile Asn Ile Ile Met  
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 Pro Ser Val Phe Gly Thr Ile Cys Leu Leu Gly Ile Ile Gly Asn Ser  
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 Thr Val Ile Phe Ala Val Val Lys Lys Ser Lys Leu His Trp Cys Asn  
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 Asn Val Pro Asp Ile Phe Ile Ile Asn Leu Ser Val Val Asp Leu Leu  
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 Phe Leu Leu Gly Met Pro Phe Met Ile His Gln Leu Met Gly Asn Gly  
                   165                  170                  175  
 Val Trp His Phe Gly Glu Thr Met Cys Thr Leu Ile Thr Ala Met Asp  
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 Ala Asn Ser Gln Phe Thr Ser Thr Tyr Ile Leu Thr Ala Met Ala Ile  
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 Asp Arg Tyr Leu Ala Thr Val His Pro Ile Ser Ser Thr Lys Phe Arg  
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 Lys Pro Ser Met Ala Thr Leu Val Ile Cys Leu Leu Trp Ala Leu Ser  
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 Phe Ile Ser Ile Thr Pro Val Trp Leu Tyr Ala Arg Leu Ile Pro Phe  
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 Pro Gly Gly Ala Val Gly Cys Gly Ile Arg Leu Pro Asn Pro Asp Thr  
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 Asp Leu Tyr Trp Phe Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu  
                   275                  280                  285  
 Pro Phe Val Val Ile Thr Ala Ala Tyr Val Lys Ile Leu Gln Arg Met  
                   290                  295                  300

Thr Ser Ser Val Ala Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr  
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 325 330 335  
 Cys Trp Ala Pro Tyr Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser  
 340 345 350  
 Arg Pro Thr Leu Thr Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu  
 355 360 365  
 Gly Tyr Ala Asn Ser Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys  
 370 375 380  
 Glu Thr Phe Arg Lys Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln  
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 Gly Gln Leu Arg Thr Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg  
 405 410 415  
 Thr Glu Ser Lys Gly Thr Val Asp Gly Thr Ala Gly Pro Gly Ser Ile  
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 Ala Thr Met Val Ser Lys Gly Glu Leu Phe Thr Gly Val Val Pro  
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 Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys  
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 Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val  
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 Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val  
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 Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu  
 545 550 555 560  
 Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu  
 565 570 575  
 Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln  
 580 585 590  
 Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp  
 595 600 605  
 Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly  
 610 615 620  
 Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser  
 625 630 635 640  
 Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu  
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 Lys

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 <213> Human

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 <212> DNA  
 <213> Mouse

<220>  
 <221> misc\_feature  
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 <223> n = A,T,C or G

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&lt;211&gt; 3357

&lt;212&gt; DNA

&lt;213&gt; Mouse

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (1)...(3357)

&lt;223&gt; n = A,T,C or G

&lt;400&gt; 18

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&lt;210&gt; 19

&lt;211&gt; 1062

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Human short form/mouse species chimeric MCH1R

&lt;400&gt; 19

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cccgataacc	tcacttcggc	aggatcacct	cctcgcacgg	ggagcatctc	ctacatcaac	120
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caccagctca	tgggcaatgg	ggtgtggcac	tttggggaga	ccatgtgcac	cctcatcacg	360
gccatggatg	ccaatagtca	gttcaccagc	acctacatcc	tgaccgccat	ggccattgac	420
cgctacctgg	ccactgtcca	ccccatctct	tccacgaagt	tccggaagcc	ctccatggcc	480
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tatgccaggc	ttatccccct	cccagggggt	gctgtgggct	gtggcatccg	cctaccaaac	600
ccagatactg	atctttactg	gttcactctg	tatcagtttt	tcctggcctt	cgcccttccg	660
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cagacagctg	acgaggagag	gacagaaagc	aaaggcacct	ga		1062

&lt;210&gt; 20

&lt;211&gt; 1269

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Human long form/mouse species chimeric MCH1R

&lt;400&gt; 20

atgtcagtg	gagccatgaa	gaagggagtg	gggagggcag	ttgggcttgg	aggcggcagc	60
ggctgccagg	ctacggagga	agaccccctt	cccaactgcg	gggcttgccg	tccgggacaa	120
ggtggcaggc	gctggaggct	gccgcagcct	gcgtgggtgg	aggggagctc	agctcggttg	180
tgggagcagg	cgaccggcac	tggctggatg	gacctggaag	cctcgctgct	gcccactggt	240
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ggcacctga						1269

&lt;210&gt; 21

&lt;211&gt; 966

&lt;212&gt; DNA

&lt;213&gt; Aequorea Victoria

&lt;400&gt; 21

tcacacgaa	taaaagataa	caaagatgag	taaaggagaa	gaacttttca	ctggagttgt	60
cccaattctt	gttgaattag	atgggtgatg	taatgggcac	aaattttctg	tcagtggaga	120
gggtgaagg	gatgcaacat	acggaaaact	tacctttaa	tttatttgca	ctactggaaa	180
actacctgtt	ccatggccaa	cacttgtcac	tactttctct	tatgggtgtc	aatgcttttc	240
aagataccca	gatcatatga	aacagcatga	ctttttcaag	agtgccatgc	ccgaagggtta	300
tgtacaggaa	agaactatat	ttttcaaaga	tgacgggaac	tacaagacac	gtgctgaagt	360
caagtttgaa	ggtgatcccc	ttgttaatag	aatcgagtta	aaagggtattg	attttaaaga	420
agatggaaac	attcttggac	acaaattgga	atacaactat	aactcacaca	atgtatacat	480
catggcagac	aaacaaaaga	atggaatcaa	agttaacttc	aaaatttagac	acaacattga	540
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catggatgaa	ctatacaaat	aaatgtccag	acttccaatt	gacactaaag	tgtccgaaca	780
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agattcatta	aaattgtatg	aataatttat	tgatgttatt	gatagagggt	attttcttat	900
taaacaggct	acttggagtg	tattcttaat	tctatattaa	ttacaatttg	atttgacttg	960
ctcaaa						966

&lt;210&gt; 22

&lt;211&gt; 765

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; GFP derivative

```

<400> 22
gtcgacggta ccgcggggccc gggatccatc gccaccatgg tgagcaaggg cgaggagctg      60
ttcaccgggg tgggtgcccac cctgggtcgag ctggacggcg acgtaaacgg ccacaagtct      120
agcgtgtccg gcgagggcgga gggcgatgcc acctacggca agctgacctt gaagtctatc      180
tgcaccaccg gcaagctgcc cgtgccctgg cccaccctcg tgaccaccct gacctacggc      240
gtgcagtgtc tcagccgcta ccccgaccac atgaagcagc acgacttctt caagtccgcc      300
atgcccgaag gctacgtcca ggagcgcacc atcttcttca aggacgacgg caactacaag      360
acccgcggcg aggtgaagtt cgagggcgac accctgggtg accgcatcga gctgaagggc      420
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cacaacgtct atatcatggc cgacaagcag aagaacggca tcaaggtgaa cttcaagatc      540
cgccacaaca tcgaggacgg cagcgtgcag ctgcgcgacc actaccagca gaacaccccc      600
atcggcgacg gccccgtgct gctgccccac aaccactacc tgagcaccga gtccgcccctg      660
agcaaaagacc ccaacgagaa gcgcgatcac atgggtctgc tggagtctgt gaccgcccgc      720
gggatcactc tcggcatgga cgagctgtac aagtaaagcg gccgc      765

```

```

<210> 23
<211> 720
<212> DNA
<213> Artificial Sequence

```

```

<220>
<223> GFP derivative

```

```

<400> 23
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ggcaagctga ccctgaagtt catctgcacc accggcaagc tgcccgtgcc ctggcccacc      180
ctcgtgacca ccttgacctc cggcgtgcag tgcttcgccc gctaccccca ccacatgaag      240
cagcacgact tcttcaagtc cgccatgccc gaaggctacg tccaggagcg caccatcttc      300
ttcaaggacg acggcaacta caagacccgc gccgaggtga agttcgaggg cgacaccctg      360
gtgaaccgca tcgagctgaa gggcatcgac ttcaaggagg acggcaacat cctggggcac      420
aagctggagt acaactacaa cagccacaag gtctatatca ccgccgacaa gcagaagaac      480
ggcatcaagg tgaacttcaa gaccgcgcac aacatcgagg acggcagcgt gcagctcgcc      540
gaccactacc agcagaacac ccccatcggc gacggccccg tgctgctgcc cgacaaccac      600
tacctgagca ccagtcggc cctgagcaaa gaccccaacg agaagcgcgga tcacatggtc      660
ctgctggagt tcgtgaccgc cgccgggata actctcgga tggacgagct gtacaagtaa      720

```

```

<210> 24
<211> 720
<212> DNA
<213> Artificial Sequence

```

```

<220>
<223> GFP derivative

```

```

<400> 24
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ggcaagctga ccctgaagtt catctgcacc accggcaagc tgcccgtgcc ctggcccacc      180
ctcgtgacca ccttcgggta cggcgtgcag tgcttcgccc gctaccccca ccacatgcgc      240
cagcacgact tcttcaagtc cgccatgccc gaaggctacg tccaggagcg caccatcttc      300
ttcaaggacg acggcaacta caagacccgc gccgaggtga agttcgaggg cgacaccctg      360
gtgaaccgca tcgagctgaa gggcatcgac ttcaaggagg acggcaacat cctggggcac      420
aagctggagt acaactacaa cagccacaac gtctatatca tggccgacaa gcagaagaac      480
ggcatcaagg tgaacttcaa gatccgccac aacatcgagg acggcagcgt gcagctcgcc      540
gaccactacc agcagaacac ccccatcggc gacggccccg tgctgctgcc cgacaaccac      600
tacctgagct accagtccgc cctgagcaaa gaccccaacg agaagcgcgga tcacatggtc      660
ctgctggagt tcgtgaccgc cgccgggata actctcgga tggacgagct gtacaagtaa      720

```

```

<210> 25
<211> 720
<212> DNA

```

## &lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; GFP derivative

&lt;400&gt; 25

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ggcgacgtaa	acggccacag	gttcagcgtg	tccggcgagg	gcgagggcga	tgccacctac	120
ggcaagctga	ccctgaagtt	catctgcacc	accggcaagc	tggccgtgcc	ctggcccacc	180
ctcgtgacca	ccctgacctg	gggctgacag	tgcttcagcc	gctaccccga	ccacatgaag	240
cagcacgact	tcttcaagtc	cgccatgccc	gaaggctacg	tccaggagcg	taccatcttc	300
ttcaaggacg	acggcaacta	caagaccgcg	gccgaggtga	agttcgaggg	cgacaccctg	360
gtgaaccgca	tcgagctgaa	gggcatcgac	ttcaaggagg	acggcaacat	cctggggcac	420
aagctggagt	acaactacat	cagccacaac	gtctatatca	ccgccgacaa	gcagaagaac	480
ggcatcaagg	cccacttcaa	gatccgccac	aacatcgagg	acggcagcgt	gcagctcgcc	540
gaccactacc	agcagaacac	ccccatcggc	gacggccccg	tgctgctgcc	cgacaaccac	600
tacctgagca	cccagtcgcg	cctgagcaaa	gaccccaacg	agaagcgcga	tcacatggtc	660
ctgctggagt	tcgtgaccgc	cgccgggatc	actctcgga	tggacgagct	gtacaagtaa	720

&lt;210&gt; 26

&lt;211&gt; 3092

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Mouse MCH1R-linker-EGFP

&lt;400&gt; 26

atggatctgc	aagcctcggt	gctgtccact	ggccccaatg	ccagcaacat	ctccgatggc	60
caggataatt	tcacattggc	gggtgagtcg	agttggagtc	ctccctcctc	cgggatgggt	120
gtggaaaaatg	ggaagggttc	acctcccaag	ccaaactgcc	tgggaaactt	tatcttacag	180
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acaagggttg	gtgacaagac	gtgaggcagg	cttgagggga	aagcttgctg	atgagtccca	360
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tcactctcgg	catggacgag	ctgtacaagt	aa			3092

&lt;210&gt; 27

&lt;211&gt; 3056

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Mouse MCH1R/EGFP direct fusion

&lt;400&gt; 27

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aacaatagcc	cgagaagggg	aaacagcagg	agatgattcc	agagacgtag	tgacccccag	1260
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&lt;220&gt;

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MCH1R-linker-EGFP

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38

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US01/08071

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(7) : C07K 14/72 , 19/00; C12N 15/62 US CL : 435/69.7, 252.3, 320.1; 530/350; 536/23.4 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/69.7, 252.3, 320.1; 530/350; 536/23.4 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) STN, MEDLINE search terms: fluores?, receptor#, green, g protein, melanin concentrating hormone receptor#		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NELSON et al. Characterization of an Intrinsically Fluorescent Gonadotropin-Releasing Hormone Receptor and Effects of Ligand Binding on Receptor Lateral Diffusion. Endocrinology. February 1999. Vol. 140. No. 2. pages 950-957, see entire document.	1-30
Y	AWAJI et al. Real-Time Optical Monitoring of Ligand-Mediated Internalization of alpha1b-Adrenoreceptor with Green Fluorescent Protein. Molecular Endocrinology. August 1998. Vol. 12. No. 8. pages 1099-1111, see entire document.	1-30
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art *G* document member of the same patent family		
Date of the actual completion of the international search 21 JUNE 2001		Date of mailing of the international search report 03 JUL 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer JOHN D. ULM  Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US01/08071

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BACHNER et al. Identification of Melanin Concentrating Hormone (MCH) as the Natural Ligand for the Orphan Somatostatin-Like Receptor 1 (SLC-1). FEBS Letters. 03 September 1999. Vol. 457. No.3. pages 522-524, see entire document.	1-30
Y	SALRO et al. Molecular Characterization of the Melanin-Concentrating-Hormone Receptor. Nature. 15 July 1999. Vol. 400. pages 265-269, see entire document.	1-30